IMPACT OF CROP LEVEL AND HANG TIME ON THE COMPOSITION OF FOUR WINE GRAPE CULTIVARS FROM THE NIAGARA REGION

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Dedicado a mi padre (q.e.p.d.) Víctor Manuel Moreno.

Te fuiste, pero te quedaste en mi corazón…

ABSTRACT

This study analyzed the use of two viticultural practices: “crop level” (half crop; HC, and full crop; FC) and “hang times”, and their impact on the composition of four grape cultivars; Pinot gris, Riesling, Cabernet Franc and Cabernet Sauvignon from the Niagara Region and wine volatile composition by GC-MS. It was hypothesized that keeping a full crop with a longer hang time would have a greater impact on wine quality than reducing the crop level. In all cultivars, a reduction of crop level induced reductions in yield, clusters per vine and crop load, with increases in Brix. Extended hang time also increased Brix related to desiccation. The climatic conditions at harvest had an impact on hang time effects. The GC-MS analysis detected the presence of 30 volatile components in the wine, with different odour activity values. Harvest time had a positive impact than crop reduction in almost all compounds.

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INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

The production of high quality wine is an important target worldwide, and the continuous increase of knowledge and new requirements from consumers cause viticulturists and winemakers to use different techniques that ensure high standards in quality and flavour in their wines. One technique is crop reduction; however, some crop control techniques substantially reduce final volumes, which negatively impact profitability.

The goal of this study was the evaluation of two crop levels and different hang time treatments in grapevines to assess their impact on composition and aroma in four *Vitis vinifera L.* grape cultivars: Pinot gris, Riesling, Cabernet franc and Cabernet Sauvignon from the Niagara region, Ontario, Canada. It was decided to experiment with different "hang times" (harvest dates) to determine whether keeping a full crop with a more lengthy in hang time might have a greater impact on wine quality than reducing the crop level.

1.1.1 Objectives

The general objectives were to assess the impact of crop level and harvest date, and their interactions, upon the yield components, berry, must, wine composition, and aroma compounds in wines of four *V. vinifera* cultivars. This study was divided into several activities: 1) Imposition of half and full crop treatments in each cultivar at veraison over two years with subsequent harvest at the typical point, three weeks and six weeks after; 2) Analysis of grape berries to determine the impact of both factors; 3) subsequent winemaking with respective analysis of must and wine; 4) identification and quantification of aroma components in each wine and determination of the impact of field treatments.

Vine treatments were imposed at veraison in a vineyard located within the Four-Mile Creek *sub-appellation* in the Niagara Region during the 2011 and 2012 vintages; grapes were hand-harvested and transported to the pilot winery at

CCOVI Brock University to be processed the same day. Samples for wine, must and berries were collected and stored in conditions that allowed their further analysis. The samples of Riesling and Pinot gris were analyzed for pH, titratable acidity (TA), Brix, (and ethanol in wine), while Cabernet Sauvignon and Cabernet franc were analyzed also for total anthocyanins, total phenols, color and hue. Wines were stored in conditions that controlled any oxidation process and contamination. Analysis of aromas in wine was carried out using gas chromatography-mass spectrometry (GC-MS).

1.2 Characteristics of cultivars

Grapes are considered one of the oldest agricultural crops and are cultivated to produce table fruits, dried fruits, juice and wine. The number of *cépages*, referenced as varieties, held in germplasm collections around the world is estimated at 10,000 (Alleweldt and Dettweiler 1994), most of them belonging to the European species *V. vinifera* (Pelsy et al. 2010). Among them, only few hundred are cultivated for commercial wine production (Truel et al. 1980). The selected cultivars were asexually propagated over centuries, yielding clones genetically identical to the parental plant as long as spontaneous mutations did not arise (Pelsy et al. 2010). Thus, traditionally, clones are attributed to a variety on the basis of many common ampelographic traits.

Varieties are constituted of clones that contain homogeneous phenotypic characters and minor differences. Propagation and identification are used for particular agronomic traits including color or flavour variation, early or late ripening, or limited productivity (Pelsy et al. 2010). However, when clones of the same variety are different enough to be grown for different wine production they are considered as different cultivars (Boursiquot and This 1999; Pelsy et al. 2010). The grapevine *V. vinifera* belongs to the Vitaceae family, is a diploid that has a small genome size, 475 to 500 Mb consisting of 19 chromosomes (This et al. 2006), found mainly in the temperature zones of the Northern Hemisphere, latitude 30º to 50º N, close to 10ºC to 20ºC isotherm, and extensively used in the global wine industry (Mullins et al. 1992). The genotypes are highly heterozygous and nearly all-modern cultivated varieties are hermaphroditic, self-fertile and out-

cross easily (This et al. 2006). For the purpose of this study four grape varieties were selected; two whites, Riesling and Pinot gris; and two reds, Cabernet Sauvignon and Cabernet franc, being varieties commonly produced in the Niagara Peninsula.

1.2.1 Pinot gris

Pinot gris is known as a white cultivar widely disseminated around the world, e.g. Germany, Alsace, northern Italy. It is known for the production of soft and gently perfumed wines, with pronounced varietal aroma and flavour, but also contains more color that most whites (Robinson 1996). Pinot gris, along with other Pinots like noir, blanc, Meunier, moure and teinteurier, belongs to the cultivar group of “Pinots”. They show primitive morphological characteristics analogous to those of the wild type *V. vinifera* subsp. *silvestris*, and are considered “archaic” cultivars (Hocquigny et al. 2004), having descended from the same genotype with only minor genetic differences (Regner et al. 2000). This group has a high degree of diversity manifested in traits such as berry color (Pelsy et al. 2010); in fact, Pinot gris is considered as a berry color mutant of Pinot noir, with whom it share gene pattern (Bowers et al. 1993).

Pinot gris commonly has gray-blue berries but sometimes produces a dark-blue skin color (Jackson and Schuster 2001) White-berried sectors can be found in the berries. This is a displacement from the L2 cell layer to the L1 which could be responsible for generating such a displacement affecting a few berries or a whole bunch on a cane, according to the development stage during which the displacement occurs (Pelsy et al. 2010).

This variety retains low to medium acidity and high levels of sugar, providing a “late harvest” character to wines. Other characteristics about this cultivar are: early ripening, good tolerance to drought conditions but only fair resistance to wet weather, moderate vigor, average yield if growing in most well drained soil types, low susceptibility to downy and powdery mildew as well as bunch rot and *Botrytis* (Jackson and Lombard 1993, Jackson and Schuster 2001).

1.2.2 Riesling

Riesling is known as a premium white grape cultivar around the world, and is cultivated in both hemispheres from warm climates like South Africa to areas with cold winters like Canada or Germany. It is an extremely robust vine, surviving in climatic extremes that would damage almost every other white wine variety (Pigott 1991). The vine is characterized for the hardiness of its wood, resistance to frost and early winter pruning (Robinson 1996). Riesling originating from the Rhine valley area (Mullins et al. 2007) is now cultivated on more than 67 000 ha worldwide.

It was mentioned for the first time in 1493 and since then more than 500 years of its propagation have passed (Regner et al. 2000). More than 80 Riesling clones are registered in Germany; the most prominent clone cultivated in several other European countries and overseas, is doubtless Gm 239 (Regner et al. 2000), but other well-known clones are Gm 94, 110, 119, 198 and Höchzuchtriesling (Jackson and Schuster 2001). Some characteristics of this cultivar are: moderate vigor, poor resistance to wet weather during flowering and ripening, but also susceptibility to sunburn. It is fairly sensitive to downy and powdery mildew but with a high risk to *Botrytis* bunch rot (Jackson and Schuster 2001). Rootstocks for Riesling have been selected according to soil type being reported SO 4 for stony, 5-C for calcareous loams and G-26 for deeper volcanic soils (Jackson and Schuster 2001).

Wine produced from this grape has an aroma described as flowery, steely, honeyed and mineral elements transmitted from each particular vineyard site where it is produced (Robinson 1996). It is also known for its high concentration in monoterpenes with averages around 1-4 mg/L (Mateo and Jiménez 2000). In the Niagara region in Canada, sensory characteristics of Riesling wines have been classified on their terroir differences; being the Escarpment Bench, Lake Plain and Lakeshore areas. Wines from the Bench have higher grapefruit, pineapple, melon and lemon/lime aromas, more acidity and greater lemon/lime flavour than the Lake Plain wines. Lake Plain wines were more diesel/petrol-like in character (Douglas et al. 2001).

1.2.3 Cabernet franc

This cultivar is more likely to be blended with other varieties like Cabernet Sauvignon or Merlot; in fact, Cabernet Sauvignon varietal character normally dominates the characteristics of Cabernet franc in blends. Nonetheless, it is grown as a varietal in regions such as the Loire, France. It is a characteristic cultivar from the group of Bordeaux, France, where it has its origin (Mullins et al. 1992), and is related to Cabernet Sauvignon (Bowers and Meredith 1997). Characteristic clones for this cultivar are: ENTAV 1, 214 and 332, with preferences for 214 over 1 for color, although problems with color are attributed more to overcropping than clone (Wolf et al. 2002). The wine characteristics are related typically as light/medium body with more fruit and herbaceous aromas than Cabernet Sauvignon (Robinson 1996).

The vine is resistant to cool inland conditions, it buds and matures more than a week earlier than Cabernet Sauvignon and is less susceptible to water stress during harvest (Robinson 1996). In cold regions, dormant buds of Cabernet franc are typically several degrees more cold-hardy than Cabernet Sauvignon buds during fall and winter, but Cabernet franc deacclimates more rapidly in the spring (Wolf et al. 2002). This variety is known for being attacked by leafroll virus, *(CLRaV) closteroviruses.* (OMAFRA 2009b).

1.2.4 Cabernet Sauvignon

Without a doubt, this is the most widespread and well-known red wine cultivar around the world. Aromatic characteristics are usually related to blackcurrant and sometimes pepper. However, local physical attributes, or *terroir*, the winemaking process and the vintage characteristics will develop particular aromatic attributes to the wine. Cabernet Sauvignon is an offspring of Cabernet franc and Sauvignon blanc (Bowers and Meredith 1997), with its origin in Bordeaux, France (Mullins et al. 1992). Mutations of this cultivar are known as bronze berry and white berry formations (This et al. 2006). Several clones have been reported with different viticultural performance like FPMS 2, 4, 5, 6, 8, 10, and 21. FPMS 6 had the greatest pruning weight, 2.8 kg/vine, and the lowest

average yield, 7.0 kg/vine, while FPMS 8 and 10 had the least pruning weight, 2.0 and 1.9 kg/vine respectively (Wolpert et al. 1995).

Grape berries from this cultivar are usually a small size, having a high seed to pulp ratio that would be make a direct impact in the final wine (Robinson 1996). In cool climates, it ripens late in the season and is characterized for its high acidity and tannin concentration. Some other characteristics for this cultivar: extremely vigorous, resistant to wet weather conditions during harvest and the ability to grow in all types of soils. Its yield is low but could be increased with correct pruning like cordon-cane (Jackson and Schuster 2001). Due to the thickness of its skin, this variety is relatively resistant to rot (Robinson 1996) but with a high susceptibility to powdery mildew.

1.3 Effect of vintage.

The effect of vintage is as important as the characteristics of *terroir*, viticultural and winemaking practices and is highly linked to the climatic conditions that are present each year. The changes in climate around the world are impacting the characteristics of grapes and wines, and making the specialists rethink their strategies when decisions of delay or early harvest must be applied. Canada, a well-known cool climate region, is not an exception; this change in climate could be beneficial (Jones & Davis 2000), and therefore a more interventionist winemaking style involving water additions, acid adjustments and alcohol reductions may be required in the future (Mira de Orduña 2010).

Among the most important climate change-related effects are advanced harvest times and temperatures, increased grape sugar concentrations that lead to high wine alcohol levels, lower acidities and modification of varietal aroma compounds (Mira de Orduña 2010). A long-term (1952-1997) climatology study was developed using reference vineyard observations in Bordeaux, France (Jones and Davis 2000). This study found that over the last two decades, the phenology of grapevines in Bordeaux has tended towards earlier phenological events, a shortening of phenological intervals, and a lengthening of the growing season. Vintage ratings have shown a general increase over the last two decades paralleling the observed phenology and composition trends.

Rankine et al. (1971) found that the year of vintage had a significant influence on pH, N, P, K, Ca, Mg, tartaric acid and TA in the juices and wines of the three grape varieties; Shiraz, Riesling and Clare Riesling. Another study with vintages with must and wine from different varieties from Alentejo sub-region, Portugal (Herbert et al. 2005), found that not only grape variety and region of production, but also vintage can influence free amino acid and amine contents of musts and wines, specifically tyramine, which was confirmed in red wines immediately after malolactic fermentation.

Pereira et al. (2006) studied the changes in metabolite fingerprints of grape berry skins of Merlot, Cabernet franc and Cabernet Sauvignon cultivars harvested in 2002, 2003 and 2004 from five geographical locations in Bordeaux, France. Many variables such as total soluble sugars, TA, nitrogen and phenolic compounds, contributed to describe grape quality. They varied strongly with genetic and environmental factors like climate, soil, and cultural practices, together referred to as *terroir*, and vintage factors. The vintage effect on grape metabolic profiles prevailed over the soil effect in this study.

The use of nuclear magnetic resonance analysis coupled with multivariate statistical data sets were used to investigate the effects of vintages on metabolic profiles of wine and the relationship between wine metabolites and meteorological data. South Korean wild grapes, Meoru (*V. coignetiae*), were harvested in the region around Gamak Mountain, between 2006 and 2007 (Lee et al. 2009). Higher levels of proline, lactic acid and polyphenols were observed in the 2006 vintage wines compared to 2007, showing excellent agreement with the meteorological data that the sun exposure time and rainfall in 2006 were approximately two times more and four times less, respectively, than in 2007.

However, vintage will not always make a difference. A study of vintage conducted by Boselli et al. (2004) with red wines from the *Denominazione di origine controllata* (DOC) in Marche, Italy, during three different vintages ranging from 1996 to 2000 found that the influence of the cultivar (or blend of cultivars) used for the winemaking was more important than the vintage year.

In a different way, a study by Fischer et al. (1999) used descriptive analysis to investigate the sensory properties of commercial Riesling wines from two vintages, five wine estates and six vineyard designations within the viticulture region of Rheingau. The analysis revealed substantial variations within the same vineyard designation and demonstrated the strong impact of the individual wine estate and vintage, being vineyard more important.

1.4 Effect of crop level

The use of crop reduction is a practice that is followed by winemakers, and is an important factor that could affect the interaction between the grapevine morphological development and physiological response (Edson et al. 1995). Manipulation of crop, due to cluster thinning postbloom or reduction in clusters after veraison, could affect the characteristics in the final wine due to changes in fruit composition. Effects of reduction of crop and overcropping; i.e. any crop level reduction applied, are well described and the consequences well understood.

Overcropping

Since the 1950s, the effect of overcropping has been studied with relevance in the production of grapes and wine around the world. Winkler (1954) pointed out that the effect of overcropping could delay the maturing of grapes. This generates an effect on the vine growth causing a lowering or depletion of the reserve materials of the vine in general and of the root system in particular. Vines with higher crop loads allocate their carbon resource to fruit production with a reduction in shoot growth, leaf size and leaf area. This change in carbon allocation is important for the development of grape constituents after veraison (Edson et al. 1995).

The accumulation of reserves in the vine itself is very slow until the fruit approaches minimal maturity. Here the effect of overcropping during the season produces a vine largely deprived of reserves. Other effects in vine response to overcropping include a reduction of yield the year following overcropping (Kliewer and Ough 1970). Alterations in fruit quality, as maturity, probably arise from the fact that changes in the different constituents of the fruit are not affected in the

same way, e.g. the rate of acid reduction remains practically constant, while the rate of sugar accumulation is greatly decreased (Winkler 1954). Also, the delay in color production can impact red cultivars; however, this is not always observed since many red wine varieties are completely colored before they attain fruit maturity. In general, overcropping can have negative effects on fruit quality, wood maturity and vine size maintenance (Edson et al. 1993). Also, an increase in crop load may lead to increased pH and lowered TA, adversely affecting color of red wines, and renders fermented products susceptible to microbial spoilage and reduction in shelf life (Morris and Cawthon 1982). An increase in cropping levels in most circumstances reduces must sugar, berry size, and pH, and increases TA, K, tartrate and malate (Boulton 1980).

Crop reduction

Effects of crop level reduction are generally an increase in Brix, anthocyanins, total phenols, and color intensity (Jackson and Lombard 1993; Mazza et al. 1999; Reynolds et al. 1994). Increased fruit quality and yield is concomitant with vine capacity due to regulation of pruning severity and fruit thinning (Weaver et al. 1961) and increase of berry weight (Freeman and Kliewer 1983). However, it was also reported that excessive cluster thinning might also result in an undesirable rise in pH mainly in places where high pH levels are an issue (Di Profio et al. 2011a)

With respect to grape quality and composition, Freeman and Kliewer (1983) studied two levels of crop (not thinned and thinned to one cluster per shoot shortly following fruit set) on Carignane grapes in California, and found a tendency towards an increase in soluble solids concentration in fruits from thinned vines. Crop thinning increased berry weight but had no effect on the rate of increase in berry weight. They pointed out that berry weight declined after reaching a maximum, probably due to moisture loss and berry shriveling which sometimes occurs late in the season. In this experiment there was not impact over TA and pH in grape juice, but an increase of soluble solids and decrease of TA at around 17º to 18º Brix. This change in the rate of decline in TA may have been due to a change in the acid composition of the berries. Tartaric acid is more

stable at higher temperatures and is degraded slower than malic acid (Boulton 1980).

In another study focused upon the effect of grape composition, Hepner and Bravdo (1985) measured the effects of crop level treatments and drip irrigation schedules on the K contents of grape leaves, must and wine of Cabernet Sauvignon. Starting with the premise that K is recognized as a factor of considerable influence on the acid balance of grape juice and wine, affecting pH, color, and fermentation processes and, ultimately, the flavour and clarity of the bottled wine (Somers 1975). K has been found as the major cation in the gradual salt formation from tartaric and malic acids in the various stages of berry development (Hale 1977). This exchange of H^+ ions in organic acids by monovalent cations practically accounts for the pH changes in the grape juice and the wine (Boulton 1980). The treatments for this experiment were three crop levels, obtained by cluster thinning right after fruit set: unthinned control (60 to 80 clusters/vine); medium crop level (40 cluster/vine retained); low crop level (20 clusters/vine retained). Some findings were: neutral salt of the tartrate was inversely related to the free tartaric acid content, but no relationship to wine quality was observed; K increased at reduced crop levels; color intensity also increased with diminishing crop levels, particularly compared to the extremely high crop loads of the full-yield treatment.

Bravdo et al. (1984) also measured the effect of three crop levels by cluster thinning imposed immediately after blooming in a high yielding Carignane vineyard of Mazkeret Batya, Israel.

A reduction of cluster number from about 60 to 40 per vine did not result in reduction of yield, since berry size and number per cluster were increased, but in the case of thinning to 20 clusters per vine, a reduction in yield was evident since the increase in berry size and number was not sufficient to compensate for the reduced number of clusters. Pruning weight of the thinned treatment was increased and so was the capacity of the vines, which is expressed here as both vegetative growth and crop yield. Cluster thinning in this experiment reduced crop load (or Ravaz index), a measurement that is expressed as yield to pruning

weight ratio, from 19 to ≈ 12 and 10, and consequently many typical overcropping phenomena, mentioned before, were eliminated and wine quality improved.

They concluded that crop loads > 12 have conspicuous effects of overcropping i.e. reduced wine quality, color quality and intensity, delayed maturation, reduced rate of sugar accumulation, must acid concentration at comparable sugar content. Another crop load value was obtained for Cabernet Sauvignon vines related to wine quality, i.e. tasting. Bravdo et al. (1985) related values > 10 of crop load with negative effects on wine quality and with apparent non significant effects with values < 10. Crop load substantially affected the dimensions of vine morphology, most importantly by reducing the leaf area available for producing photosynthates (Edson et al. 1995).

Ough and Nagaoka (1984) reported that grapes of Cabernet Sauvignon from thinning treatments, two weeks after bloom, resulted in higher pH and a trend towards a higher TA as well as higher °Brix at the same harvest date. Reynolds et al. (1994) found that reduction in crop levels in Pinot noir grapes imposed at bloom had a slight reduction in yield, increase in cluster weight with more berries/cluster and increase in berry weight. Results for juice composition were less consistent, particularly between years; decreasing crop level increased berry °Brix in three of four seasons and in the four-year means, and increased TA and pH slightly in just one of four years; this discrepancy between pH and ºBrix could be due to the canopy density.

Another aspect in berry composition related with crop level is the production of arginine and proline; the latter is associated with plant senescence and drought stress (Kliewer and Ough 1970). Crop thinning tended to increase the level of arginine in berry juice in Carignane vines, but differences between thinned and unthinned vines were not consistent, the overall average proline concentrations in the fruit from thinned vines were also higher (Freeman and Kliewer 1983). The concentration of arginine greatly increased with fruit maturation and as with arginine, the concentration of proline greatly increased with fruit maturity. Kliewer and Ough (1970) found a relationship between these two amino acids in Thompson Seedless grapes affected by crop level; here, the

ratio of arginine to proline decreased with fruit maturity indicating that the concentration of proline increased at a faster rate than that of arginine during the latter stages of fruit ripening. Also as crop weight decreased; as a result of cluster removal, the arginine/proline ratio decreased. As crop size decreased relatively, there was less competition for photosynthates and nitrogenous compounds, and therefore a greater supply of these substances available for the remaining fruits (Kliewer and Ough 1970).

Reynolds et al. (2007) measured the impact of different cluster thinning timings from bloom to veraison on the sensory and berry/must/wine composition of Chardonnay Musqué. They found that thinning decreased yields and clusters per vine regardless of timing, but both berry and cluster weights decreased greater as time of thinning was delayed, suggesting yield compensation in earlier treatments. The results for thinning at/after the early stage led to higher Brix, pH, and potentially volatile terpenes relative to the control, TA decreased with later thinning, and free volatile terpenes increased in some thinning treatments. Very few sensory differences were found among the viticultural treatments despite differences in chemical composition.

Effect on wine

The effect of crop level on some berry and must constituents are transferred to the wines. An indication of potential wine quality is reflected by lower pH, higher tannin, higher extract, higher color, and high total acidity, all of which help to produce wines of better keeping quality with a better potential for aging (Weaver et al. 1961). Mazza et al. (1999) measured the effect of cluster thinning at bloom and at veraison on the anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia, and found that cluster thinning at veraison generally resulted in higher wine phenolic contents than in controls, but with differences depending on cultivars. In fact, they suggested that variations in results for Cabernet franc suggested that other factors besides viticultural treatment could have a significant influence on grape development in this variety. Conversely, crop level had no effect on the

concentration of anthocyanins in berry skins but there was followed with a reduction in color of the final wine (Freeman and Kliewer 1983).

Following with the work of Hepner and Bravdo (1985) in Carignane and Cabernet Sauvignon must and wine, they found that pH was not affected by the crop level treatments, probably because the cation and anion concentrations fluctuated in parallel. They also indicated that wines with high K^+ and pH levels acquired a dull color, while wines with a lower pH attained a bright red color suggestive of good quality.

Two different effects in wine were found depending on the variety; for Carignane (Bravdo et al. 1984) the poor wine color of the unthinned treatment was associated with high crop load generating overcropping effects like; high K^+, and low TA at comparable ºBrix levels, whereas in Cabernet Sauvignon (Bravdo et al. 1985), the best color was in unthinned treatment and was associated with high crop load, low pH, low K^+ and high TA. The limiting factor proposed in Carignane for this experiment was anthocyanin biosynthesis due to overcropping.

The quality of wines produced from the unthinned treatment in high yielding Carignane grapes was inferior to that of the other two treatments where cluster thinning was applied (Bravdo et al. 1984). They found also that volatile acids were high in unthinned wines in two years. Amino acid content of the must was low in the unthinned musts which produced inferior wines, while higher amino acid contents, especially arginine and proline, increased the fermentation rate and produced a more intensive aroma in the thinned treatments, consistent with the findings of Bell et al. (1979). However, Bravdo et al. (1985) described that quality tended to be slightly better in wines made with Cabernet Sauvignon of the unthinned treatment than in wines from reduced crop imposed after bloom. Another discrepancy was found in Cabernet Sauvignon from Ough and Nagaoka (1984), who described that the quality of the wine slightly increased by thinning in two of the three years of experiment. Here thinning of the clusters had a minimal effect on ripening time, must analysis, wine analysis, and wine aroma, but increased wine quality with thinning.

Reynolds and Wardle (1989) described the effect of cluster thinning over the varietal aroma intensity in Gewürztraminer wine. The tasters found no major differences in varietal intensity; although cluster thinned wines were scored highest in this category, corresponding to the high potentially volatile terpene levels in the fruit from that treatment. Reynolds et al. (1996) measured the crop level effects on Pinot noir from Oregon and British Columbia and found that reducing crop level increased ethanol and anthocyanins in Oregon wines, but British Columbia half-crop wines had higher TA and pH (they proposed that this could occurred due to clusters retained were shaded, due to larger leaves, more lateral shoot growth, or higher overall vine vigour), high anthocyanins, and ethanol. In a sensory evaluation, the tasters were able to distinguish between canopy treatments and/or crop levels in wines using descriptors like vegetative (aroma and flavour), black pepper aroma, chocolate aroma and astringency. Reducing crop level increased color, currant aroma, astringency, and intensity of finish independent of canopy treatment.

Di Profio et al. (2011b), found that cluster thinning treatments on Cabernet franc, Cabernet Sauvignon and Merlot had the highest wine anthocyanin and phenol concentrations and the highest color intensities with respect to control, with an increase in pH and a reduction in TA, the last being directly correlated with the results of TA on berry and must as well as color and total anthocyanins (Di Profio et al. 2011a).

1.5 Effect of hang time

Overcropping is known for a link in a delay in fruit maturation and direct effect on the quality of the grapes and the final wine. Crop load reduction can be beneficial with direct consequences to yield and wine volume. Therefore, the fundamental questions are: what if it is decided to hang the fruit for longer periods of time without any crop reductions to obtain higher volumes of well-ripened fruit? Could the quality of grapes and wine be compromised for an extension in the period of hang time? To find some answers it is important to understand the effect of hang time. Moreno et al. (2008) described extended ripening as the period of time that fruit is left on the vine beyond the time needed

for an acceptable sugar concentration. This definition could be linked to the vines that are adjusted in crop level. In the case of overcropped vines, this extension could be used to reach the acceptable maturity for harvest.

The effect of hang time is partially linked to the reduction in berry weight that is related to loss of water and increase in sugar content. This has an impact on all aspects of grape composition that are directly proportional to the composition of the final wine. Increases in sugar can lead to changes in flavour and development of non-aromatic precursors that could be released in the final product.

Climatic conditions also impact grape composition and berry weight during extended hang time. The amount of rainfall during the harvest period, as well as solar radiation and temperature, affect photosynthetic metabolites. They can make an important difference between vintages and final quality of wine including the action of some spoilage microorganisms.

The first premise of the effect of hang time is that grapes that hang on the vines beyond the normal time to attain a higher level of sugar become physically overmature. Such grapes are more susceptible to handling and transportation injury (Winkler 1954). In the case of wine grapes, this process is generated by dehydration and is sometimes promoted to increase sugar content by concentration (Constantini et al. 2006).

Dehydration

Wines made from increasingly dehydrated grapes tend to resemble the composition and flavour profile of wines made from grapes left on the vine, i.e. with extended ripening (Moreno et al. 2008). Therefore, the effects of dehydration of grapes could be used to understand the effects of hang time. Water loss can occur during the over-ripening process of grapes on the vine, in the presence (or absence) of noble rot (*B. cinerea*), or off the vine in the field, or indoors under fully or partially controlled dehydration conditions (Chkaiban et al. 2007)

The use of postharvest dehydration of grapes for wine production has shown that in addition to sugar concentration, phenolics and aroma compounds are either concentrated or produced (Bellincontron et al. 2004; Constantini et al.

2006). Differences between wine ethanol concentrations have been associated with the differences in soluble solids (Moreno et al. 2008). Bellincontron et al. (2004) reported the use of passing air through a tunnel to generate dehydration of Malvasia, Trebbiano and Sangiovese grapes for wine production. Incremental changes in soluble solids and TA were detected in comparison with the control. Malic acid was consumed in the first step of dehydration but an increase in the dehydration ratio could mask the malate loss.

Sugar also can be synthesized by malic acid in the last step of the slow grape dehydration process (Amati et al. 1983; Corte et al. 2001). Total phenols and anthocyanins almost doubled in tunnel-treated Sangiovese berries, and volatile compound analysis revealed a higher ethanol concentration in all tunnel-treated grapes but a lower concentration of ethyl acetate and acetic acid (Moreno et al. 2008). Pérez-Magariño and González-San José (2004) found that wines made from more mature grapes of Tinto fino and Cabernet Sauvignon had generally higher free anthocyanin content. During aging, the decrease of the free anthocyanins and flavanols took place in conjunction with an increase in the levels of the anthocyanin derivatives or “new pigments”, which are responsible for maintaining color intensity and adding violet hues in aged wines.

Constantini et al. (2006) reported two metabolic stress stages during the postharvest drying of Malvasia grapes, a first metabolic stress response up to 11.7% of bunch weight loss and a second stress response beyond 19.5% of weight loss. Lipoxygenase activity, proline and abscisic acid rapidly increased when grapes reached 11.7 % of weight loss but this was followed by a gradual decline (Constantini et al. 2006). At the same desiccation level, C_6 compounds, e.g. hexanal, hex-1-enol, and (E)-hex-2-enal, reached a peak in concentration, whereas ethanol and acetaldehyde increased with the increase of alcohol dehydrogenase and proline and successively decreased, and ethyl acetate increased (Zamboni et al. 2008).

When a loss of water of 0.5% occurs in grapes, the cell wall enzyme activity is increased, and a further increase of water loss accelerates respiration and ethylene production. At the same time, a change or reduction of volatiles and

polyphenol levels is observed, not only due to concentration but due to change of metabolism (Bellincontron et al. 2004; Constantini et al. 2006; Zamboni et al. 2008). The drying of fruit also generates shrinkage, which modifies the shape and dimension of products affecting the mass transport phenomena (Kays 1997; Wang and Brennan 1995).

Wines made from dehydrated grapes contain more terpenes and norisoprenoids (β -ionone, β −Damascenone) compared to wines made from the original fruit (Moreno et al. 2008). Carotenoid profiles declined in Gewürztraminer grapes dried in a thermo-conditioned tunnel, slightly increasing at the end of the experiment in both samples (tunnel and control), with the decline more rapid in the control grapes (Chkaiban et al. 2007). The oxidation of carotenoids during the dehydration process is important for the formation of specific volatiles. It can be useful as a stress marker since they play an important role in protecting the cell against stress conditions. It has been reported that they decrease during grape ripening (Razungles et al. 1996, Oliveira et al. 2003). An AFLP-transcriptional profiling analysis in grape showed that two transcripts with homology to a limonoid UDP-glucosyltransferase were induced during the post-harvest drying (Zamboni et al 2008). In citrus fruits, limonoid UDP-glucosyl transferase catalyzes the conversion of bitter tasting limonene to limonoid glucoside. However, there is no evidence for the presence of limonene in grape berries, but it is possible that this gene is involved in the modification of other terpenes or in the production of secondary metabolites and hormones (Kita et al. 2000).

Pathogen infection

As was mentioned before, grapes that are hung during long periods of time will be more susceptible to handling and transportation injury as well as infections due to microorganisms like *B. cinerea.* These molds produce polyalcohols (polyols) in high concentrations such as glycerol, arabitol and mannitol (Sponholz 1993). This occurs when injury in a berry allows the penetration and infection for this fungus, generating browning and shriveling in white cultivars or reddish-brown in red cultivars, appearing as a greyish-tan conidia first seen in tufts or along splits in berries (OMAFRA 2009a). Another

effect produced as a result of the presence of *B. cinerea* is the drying out of berries due to shrinking. A loss of water occurs by uptake of microorganisms and evaporation, causing a concentration of ingredients in grape and water stress due to the high production of polyols (Sponholz 1993). Excessive humidity favours the growth of *B. cinerea* in the form of grey rot that causes a great decrease in grape quality. When the development of the fungus is inhibited and the grapes have lesions, the proliferation of other microorganisms leads to total deterioration of the grapes (Donèche 1993)

Sour rot is a disease characterized by a typical and easily recognized phenomenon of browning and disintegration of the internal tissues. It is normally detected by a detachment of the rotten berry from the pedicel, and a strong ethyl acetate smell (Guerzoni and Marchetti 1986). In wine, *Zygosaccharomyces bailii* has been recovered as the spoilage species at the end of fermentation. But, a high diversity of species, a total of 22 Ascomycetous fungi, could be present in damaged grapes with sour rot (Barata et al. 2008).

An analysis of sour rot on Riesling grapes from a Virginia vineyard detected an increase in ºBrix, TA, tartaric acid, glycerol, gluconic acid and glucose to fructose ratio with a reduction in berry weight in those grapes infected (Zoecklein et al. 2010). No influence was detected in that experiment for components like pH, acetic acid, ethanol or laccase activity as well as free and potential volatile terpenes. In selected free aromas, rot reduced free geraniol, nerol and linalool concentrations and increased trans-furan linalool oxide, benzyl alcohol, 2-phenethyl ethanol, 2-methy-1-propanol and 3-methyl-1-butanol (Zoecklein et al. 2010).

1.6 Aromatic compounds in wine and must

In wine, > 800 compounds have been identified in their volatile fraction (Ortega-Heras et al. 2002). Some of these compounds can be associated with varietal characteristics or are generated during fermentation, while others are considered undesirable when they occur (Bakker and Clarke 2012). The odour thresholds for target aroma compounds found in grapes such as monoterpenes and norisoprenoids, tend to be lower than other classes of flavour and aroma

compounds, e.g. esters, alcohols, aldehydes and ketones). This is also the case for methoxypyrazines, which are considered much more odour active (Dunlevy et al. 2009).

Within the exciting world of wine, the wine flavour plays a very important role in the quality of final product. The word “flavour” usually indicates the combination of smell, or odour, and taste. However, “tasting” is the term used to assess the sensory properties of wine. Therefore flavour is defined as the combination of taste and perception of the volatile compounds present during tasting and can be considered as due to both volatile and non-volatile compounds. Such volatile compounds are usually of low molecular weight, < 300 Da, and then vaporize readily at room temperature. They give an odour sensation when they reach the olfactory epithelium, dissolve into the mucus and bind with olfactory receptors (Francis and Newton 2008).

Aroma compounds become part of the wine mix by different sources. Grape sugars, from which the fermentation process releases as prime metabolites ethanol and CO_2, can also yield secondary metabolites like esters, acids and high alcohols. Non-volatile grape derived precursors, which could also be released by enzymatic action by bacteria and yeasts, such as monoterpenes, norisoprenoids and some thiols. In addition, secondary metabolites from the action of malo-lactic bacteria, produce some esters and diacetyl (Borneman et al. 2012). Rapp (1998) used a similar classification adding maturation bouquet, which is caused by chemical reactions during maturation of wine in the bottle.

In the berry, the aromatic compounds are distributed in the flesh and skins and could be accumulated at different stages of berry formation and berry ripening depending on the type of component (Kennedy 2002). Chemically, aroma compounds in grapes could be present in two general forms; as free volatile compounds, which are generated through the action of endogenous enzymes, and glycosidically-bound volatile compounds that are consider as latent aglycones pools that would be source of wine flavour and aroma. These aglycones are linked to three different sugars: β -D-apiofuranose, α -L-

arabinofuranose, and α-L-rhamnopyranose. Also the aglycone moiety could be linked to β-D-glucopyranose as reported by Sarry and Gunata (2004).

Terpenes

Two big groups of compounds derived from grapes have an impact on the wine where they are present. These are the terpenes and norisoprenoids. Terpenes are varietal or essentially primary aromas like muscat or floral aromas that persist through the vinification process. These compounds are considered aroma precursors and are located mostly in the skin (Bayonove et al. 1993).

The hydrocarbon monoterpenes ($C_{10}H_{16}$) are the most important. At the top of this group can be found linalool, geraniol and nerol, together with the pyran and furan forms of linalool oxides (Mateo and Jiménez 2000). They classified grapes based on their terpene concentration. Muscat varieties with 6 mg/L, e.g. Muscat of Alexandria, Gewürztraminer and Canada Muscat, non-muscat aromatic varieties with 1-4 mg/L, e.g. Riesling, Sylvaner, Kerner, and neutral varieties where the concentration of terpenes is not important for the aroma, e.g. Cabernet Sauvignon, Bobal, and Semillon.

These compounds are biosynthetically formed from mevalonic acid, “mevalonate”, via the mevalonate pathway located in the cytosol, and are classified as free volatile terpenes, i.e. odour-active molecules, and potential-volatile terpenes, i.e. polyols and glycosidic precursors that have non-odour activity and therefore hydrolysis must be carried out to release the free aroma molecule. The β-glucosidase is the key enzyme in flavour release and is the most abundant with glycosidase activity in grape berry as well as vine leaves. It is distributed throughout the berry but the skin is richest in activity (Sarry and Günata 2004). *Saccharomyces cerevisiae*, involved in alcoholic fermentation, displays low levels of α-arabinofuranosidase, α-rhamnosidase, and β-glucosidase activities, the latter being most abundant (Delacroix et al. 1994).

Other yeast genera such as *Dekkera*, *Debaryomyces*, *Kloeckera*, *Hansenula* and *Candida* are able to synthesize β-glucosidase when cultured on a suitable culture medium (Leclerc et al. 1987). Günata et al., (1985) found that

grape juice produced from grapes infected with *B. cinerea* was enriched in α-arabinofuranosidase, α-rhamnosidase, and β-glucosidase activities. These grapes could be also contaminated with glucono-δ-lactone, which is a strong inhibitor of β-glucosidase activity (Heyworth & Walker 1962).

Some viticultural and winemaking practices are known for having an impact on the concentration of terpenes in grapes and wines. Reynolds and Wardle (1989) found increases in free and bound terpenes in Gewürztraminer that were associated with fruit exposure. Macaulay and Morris (1993) similarly found increases in terpene concentration during veraison in Golden Muscat exposed to more sunlight compared to those that were partially shaded. Wines produced from exposed and shaded treatments were different in potential volatile terpenes, with almost double in wines from exposed clusters compared to shaded clusters. Bureau et al. (2000) pointed out that direct sunlight on grapes can cause stress either by dehydration or temperature increases. Reynolds et al. (2006) measured the response to water stress duration in Gewürztraminer grapevines with deficits imposed at post bloom, lag phase and veraison. They found that decreasing duration of water stress increased free volatile terpenes at harvest, as well as increasing potentially volatile terpenes at both veraison and harvest. Palomo et al. (2006) found an increase in the concentration of free and glycosidically bound terpenes in wine with skin contact made with Muscat à Petite grains grapes from La Mancha region in Spain.

Norisoprenoids

Norisoprenoids are a group of volatile compounds formed by C_{13} precursors that are present in grape and wine in low concentrations. They are synthesized mostly from stage I and 2 of grape development until veraison, then degradation takes place until end of maturity, some of which subsequently became glycosylated (Baumes et al. 2002; Mendes-Pinto 2009). The principal precursors are β-carotene (represents 85%) and lutein (Mendes-Pinto 2009). These are synthetized in plastids; therefore the C_5 isoprene unit is formed via the methyl-erythritol-phosphate (Dunlevy et al. 2009). These compounds usually are

found as glycosides that represent a pool of flavour reserves in grapes. They are known as ubiquitous, adding nuances to different cultivars across different regions.

Chardonnay and Shiraz are well known for their norisoprenoid character. Carotenoids are degraded chemically, i.e. temperature, light, oxygen, and enzymatically; i.e. deoxygenases with an initial dioxygenase cleavage. Enzymatic transformation of the primary cleavage product gives a non-aromatic intermediate. Acid catalyzed conversion of these non-aroma intermediates lead to the aroma compounds (Mendes-Pinto 2009).

Some representative compounds for this group are: β-Damascenone, representative in Chardonnay with a rose-like or apple or honey odour- β-Ionone is found in Riesling grapes and can be found in considerable amounts in brandies, with violets but also cedar wood and soft strawberries odour (Dunlevy et al. 2009). Vitispirane, formed slowly during aging, has a cis-isomer with a chrysanthemum flower-fruit odour, whereas its trans-isomer has an exotic fruit-like aroma. The compound 1-(2,3,6-trimethylphenyl)buta-1,3-diene or TPN was identified in white wines (Cox et al. 2005a,b), and has flowery, fruity and earthy-wood undertones. The well-known 1,1,6-trimethyl-1,2-dihydronaphtalene or TDN, forms during the ageing some wines, especially Riesling, which develop a strong petrol-like or kerosene-like aroma. It is more apparent in wines from warm regions than colder ones.

Other aroma compounds

Volatile compounds found in grapes, musts and wines are classified in groups. This classification will be used further in Chapter 3: hydrocarbons, alcohols, carbonyl aldehydes, carbonyl ketones, acids, esters, ester lactones, bases, S-compounds, acetals, ethers, halogens, nitriles, phenols, furans, epoxides and anhydrides (Rapp 1998). Sensory differences could exist between grape varieties. However, wines will share the majority of these compounds. Here, the variety-specific compounds will make the difference. Most odour-active compounds could have low concentrations, but they have also very low sensory thresholds (ng/L) that would generate a large impact on the overall grape/wine

aroma (Polaskova et al. 2008). Avakyants et al. (1981) described that ethyl acetate, isoamyl acetate, ethyl caproate and caprylate, isobutyl and isoamyl alcohols and acetaldehyde as the compounds that can contribute to the basic odor of the wine, while the others are considered modifiers of the basic odour.

The fermentation process will generate a large group of aromatic compounds that will modify the characteristics of final wine. *S. cerevisiae* leads to the formation of many alcohols, e.g. ethanol, C_3-C_5 straight chain alcohols, *n*-alcohols, and 2-phenylethyl alcohol and esters (Polaskova et al. 2008). *Oenococcus oeni*, responsible for malolactic fermentation, can generate high concentrations of diacetyl, i.e. 2-3-butanedione, which contributes to a buttery aroma (Bakker and Clarke 2012). The oxidation process generated by the action of chemical and microbiological processes leads also to the generation of many aromatic compounds that could be either desirable or not in wine, i.e. acetaldehyde contributes to nutty sherry-like aroma desirable in aged wines and sherries, or acetic acid, with a vinegar aroma (Polaskova et al. 2008).

Esters

Considered as secondary aromas, fatty acid esters are formed through the actions of yeasts, the metabolic pathway begins from acetyl-CoA as the key component, which thereafter leads to esters, whose acid moiety has an even number of carbon atoms (Nykänen 1986). In general, it is recognized that lower aliphatic esters could show fruity notes, e.g. tropical fruits, banana, pineapple, apple, pear, whereas higher homologues tend towards wax and soap character (Bakker and Clarke 2012). Different esters could be found in the literature depending on the cultivar, generally divided between red and wine wines in mg/L. Bakker and Clarke (2012) summarized in a list (mg/L): ethyl acetate 4.5 to 180 for white and 22 to 190 in reds, ethyl butyrate 0.04 to 1 in white to 0.01 to 0.2 in red, ethyl isobutyrate 0 to 0.6 in white and 0.03 to 0.08 in red, ethyl caproate in white 0.06 to 2 in white and 0.06 to 0.13 in red, ethyl caprylate from 0.4 to 5.1 in white and 1 to 6 in red, ethyl palmitate present in white between 0.10 to 0.85, diethyl succinate in whites 0.1 to 1.4, hexyl hexanoate from trace to 1.3 in white, 2-phenyl-ethyl ethanoate from trace to 5.10 in white.

Aldehydes

Methyl ketones and aldehydes, within the group of carbonyl compounds, are formed by microbially induced lipid oxidation; initiated by lipases, hydrogen peroxide produced by microorganisms, and/or lipoxidase-like activity (Reineccius 2006). Aldehydes present in grapes or juice are only detectable in wine at early stages of fermentation (Rapp and Mandery 1986). Acetaldehyde is a common aldehyde present in some wines, around 100 mg/L (Bakker and Clarke 2012) and 42.5 to 76.5 in aged wines (Cullère et al. 2004). Above its threshold and in free form, acetaldehyde is usually regarded as an off-odour. Although also fruity in low levels, in higher levels it can be pungent and nauseating (Bakker and Clarke 2012). Another aldehyde is benzaldehyde; with a bitter almond aroma that could be a potential defect in wine but is characteristic in some cultivars like Gamay (Bakker and Clarke 2012). Hexanal is reported as has a cut grass or herbaceous aroma, nonanal has a soap-like metallic odour, decanal has a soapy, citrus-like aroma and phenyl-ethyl aldehyde (phenyl-acetaldehyde) reported as has a floral, rose, honey odour.

Alcohols

Alcohols become important compounds of the aroma in wine when they are present in high concentrations, i.e. mg/L, or unsaturated. They are produced via primary metabolic activity of microorganisms like yeasts or reduction of a carbonyl to the corresponding alcohol (Reineccius 2006). Fusel alcohols, larger metabolites than ethanol, could be formed by carbohydrate or amino acid metabolism. For amino acids, the alcohol formation could be produced by transamination, decarboxylation and reduction, or oxidative deamination followed by decarboxylation and reduction, while production from carbohydrate follows the Embden-Meyerhof-Parnass pathway to pyruvic acid (Reineccius 2006). Fusel alcohols have a characteristic pungent odour and at higher concentration, i.e. >300 mg/L, lead to negative quality factors, but in lower concentrations could add desirable aspects to the final wine (Bakker and Clarke 2012). Hexanol has leaf-grassy aroma when it has a concentration between 2.1 to 13.2 mg/L in young red wines (Guth 1997). Isobutyl alcohol has an ethereal/fruity odour with different

concentrations depending on the type of wine (Francis and Newton 2008). Isoamyl alcohol has a whisky/malt aroma in concentrations also depending on the type of wine, while 2-phenyl ethyl alcohol has a honey/rose/spice/lilac aroma.

Acids

Several acids have been identified in wine. They have different sources, like microorganism production or deamination of amino acids (Reineccius 2006), but just 14 are volatile liquid substances (Bakker and Clarke 2012). Acetic acid, with a sour or pungent odour, can be found from 69.1 to 313.3 mg/L in red wines and 30 to 489.3 mg/L in white wines (Escudero et al. 2004). Octanoic acid, with a sweet cheese odour, is found between 0.5 to 4.6 mg/L in red wines and 4.9 to 13 mg/L in white wines (Cullère 2004, Escudero et al. 2004, Lopez et al 2003). Decanoic acid with a rancid/fat odour is found between 0.06 to 0.8 mg/L in young red wines (Francis and Newton 2005) and 0.7 to 2.1 mg/L in young white wines (Escudero 2004; Lopez et al 2003).

There exist more groups of aromatic compounds in wines such as volatile phenols, e.g. vinyl-4-phenol or vinyl-4-guaiacol, Pyrazines, sulfur compounds and some compounds related with ageing can also be significant. However, they are not included here due to their lack of presence during the wine analysis in GC-MS in this experiment.

1.6.1 Analysis of volatile compounds aroma in wine and must

The chemical and sensory measurement of wine flavour is an important area of wine analysis, their measurement will help in the understanding not only at what levels they exist, but also what is the impact over the perception and quality of wine.

It has been more than a half a century since GC-MS, was developed. It has helped in the addition of hundred of chemicals responsible for the aroma in horticultural crops. This technique has been used for recognition and measurement of target compounds in mixtures like wine. Some examples of the use of this technique are the recognition of aroma thresholds, the distinction between odour and non-odour active compounds, the obtaining of a partition coefficient (which means that some compounds must partition from the liquid to

vapour phase in order to be detected) and recognition of other potential compounds (Rusjan 2010; Ferreira and Cacho 2009).

1.6.2 Odour active values

Odour activity values (OAVs) are useful measures to assess the relative importance of individual chemical compounds present in a sample. It is calculated as the ratio between the concentration of an individual compound and their perception threshold (Vilanova and Sierio 2006). Reports of the calculation of OAVs indicate differences in the way that thresholds are obtained. This is an important factor to take in to account when analysis of impact aroma compounds are obtained. For example, Grosch (1993) based his reports upon threshold values on an absolute value in pure water. However, it is known that interactions in a matrix occur, e.g. compounds found in wine like alcohol, could affect the aroma perception and change the threshold values. Guth (1997) estimated OAVs taking in to account the influence of ethanol with the use of a mix of water/ethanol (90+10 v/v) to determine thresholds of each compound. Ferreira et al. (2000) determined olfactory thresholds diluting each standard in synthetic wine prepared with ethanol 11% v/v, glycerine and tartaric acid with 7 g/L and 5 g/L respectively, with pH adjusted to 3.4.

Usually it is considered that a component has a relative aromatic importance when the ratio between its concentration and aromatic threshold is ≥ 1. However, a compound that has a value < 1 still might contribute to the aroma of a wine due to the additive effect of similar compounds with similar structure and odour.

Conversely when it has a value > 1 it does not guarantee that it will be perceived in the wine (Francis and Newton 2008).

1.6.3 Volatile aroma compounds analyzed by GC-MS

For the analysis of aromatic compounds in wines in this experiment, sorptive stir bar extraction (SBSE) technique was used. The SBSE technique is derived from the solid-phase microextraction (SPME), and used in wine aroma analysis as a method for rapid and solvent-free extraction. Hayasaka et al. (2003) compared this method (SBSE) versus SPME in the analysis of 100

aromatic constituents in Cabernet Sauvignon wines, finding higher recovery for in SBSE with lower detection and quantitation levels. Alves et al. (2005) compared HS-SPME, headspace analysis, with SBSE in Portuguese Madeira wines, and found differences in concentration for trace and ultra traces compounds when they extracted the samples in SBSE.

The SBSE, called Twister (commercially known as Gerstel), is made of a metal stir bar, inserted in a glass jacket. This stir bar is coated with polydimethylsiloxane (PDMS), an adsorbent polymer that has the capacity to extract hydrophobic compounds (Nongonierma et al. 2007). Due to the higher polymer amounts used in SBSE, volumes from 50 to 200 μL, more volatiles are extracted compared to SPME, 50 to 250 times more (Polaskova et al. 2008). Consequently, aroma molecules present in a sample at low concentrations are extracted in a quantity sufficient to be detected (Nongonierma et al. 2006). The stir bars are introduced into an aqueous solution and extraction takes place during stirring. Extraction conditions could vary between time and speed of magnetic stirrer plus the conditions used for analyte desorption. The use of sorptive stir-bar thermal desorption conditions could be manipulated as well as the GC for optimization of analysis. Since SPME is an extraction technique by immersion, and due to the relative high amount of absorbent, a high recovery can lead to overloaded chromatograms with broad or distorted peaks that will need further optimization of GC conditions (Demyttenaere et al. 2003; Polaskova et al. 2008).

For the identification of volatile compounds, even at trace levels, GC-MS is used. It allows identification of chemical constituents without the necessity of producing a pure isolate, but without an opportunity to make sensory observations about these compounds (Acree et al. 1984). Once the volatile fraction is extracted a number of complementary steps must follow: 1) *Separation*, occurring in a specific column containing a stationary phase where the analyte is adsorbed directly onto solid particles and transported to a detector (Harrys 2001). For separation of volatile fractions in a complex of medium to low polarity with similar structure and physiochemical characteristics, capillary GC

makes the best choice for volatile fraction analysis (Rubiolo et al. 2010); 2) *Identification*, usually done by GC-MS that combines GC; e.g. Kováts indices, linear retention, relative retention time, locked retention times, and MS data. Volatile compounds are transformed into ions in an ionization chamber. In a flame ionization detector (FID) the eluate is burned in a mix of H_2 and air. Carbon atoms produce CH radicals, except carbonyl and carboxyl, which produce CHO^+ ions (Harrys 2001). The mass analyzer sorts and separates the ions according to their mass to charge ratio (*m/z* value). Then, they are passed to the detector systems to measure their concentration. The results are displayed on a chart called a mass spectrum (Banerjee and Mazumdar 2012); 3) *Quantification*, with the use of pure and internal standards. Zalacain et al. (2007) described the use of synthetic wine with several pure standards for quantification of volatile aroma in six commercial monovarietal white wines from Extremadura, Spain. They diluted first each standard in pure ethanol and then diluted in the synthetic wine, prepared with 12% v/v ethanol, 5 g/L tartaric acid, and pH adjusted to 3.6 at different concentration levels to create calibrations for quantification.

1.6.4 Aromatic component in selected cultivars

For the white cultivars, Pinot gris and Riesling, the important volatile compounds found in these cultivars are monoterpenes. Terpenes as mentioned before, can be used to classify different cultivars. Terpene profiles are useful for the separation of genuine Riesling wines from other so-called Rieslings (e.g. Welschriesling, Kap Riesling, Emerald Riesling) but not produced from grapes of the variety Riesling (Rapp 1998). Pinot gris, also known as Ruländer, is considered part of the Silvaner-type group including Silvaner itself and Pinot blanc, also known as Weißburgunder, with a more neutral bouquet (Rapp and Mandery 1986). Some important compounds found are: linalool (1.0 - 19.4 μ g/L), hotrienol (3.3 – 14.7 μ g/L), terpineol (3.2 μ g/L), citronellol (0.4 – 1.0 μ g/L), and geraniol (0.8 – 3.2 μ g/L) (Rapp 1998). The difference from cooler to warmer regions could be observed in terms of monoterpene profiles. The intensities tend to be lower in the warmer climate than cooler, and consequently the sensory impact is different as well (Marais et al. 1992). The concentration of

monoterpene alcohols decreases in presence of *B. cinerea*, while the content of monoterpenediols increases significantly (Rapp and Mandery 1986). Concentrations of carotenoid degradation products in these cultivars include Damascenone at approximately 0.7 to 9.4 μ g/L, and β -ionone at approximately 0.11 μ g/L.

Some volatile thiols generated during fermentation and previously identified in Sauvignon blanc wines have also been identified in Riesling and Pinot gris wines. These include 4-mercapto-4-methylpentan-2-one (4MMP), 4-mercapto-4-methylpentan-2-ol (4MMPOH), 3-mercapto-3-methylbutan-l-ol (3MMB), 3-mercaptohexan-l-ol, (3MH) and 3-mercaptohexyl acetate (A3MH). They are reported as responsible for some particular nuances in these wines like green bell pepper, asparagus, grassy, gooseberry, box tree, broom, black currant, etc. (Tominaga et al. 2000).

Some other compounds produced after fermentation that have also been reported for these cultivars are acetates such as isobutyl, isoamyl, hexyl, 2-phenylethyl and propanol mono acetate, some esters such as diethyl succinate and malate as well as monoethyl succinate (Rapp and Mandery 1986). A specific ester present in these wines, ethyl acetate, in levels < 50 mg/L could generate pleasant odours, but turn into vinegary notes when the concentration reaches 150 mg/L (Rapp and Mandery 1986).

For red cultivars, such as Bordeaux-type wines, the concentration of monoterpenes does not characterize these varieties, but other compounds have been found over odour active concentration thresholds; ethyl octanoate, β -damascenone, ethyl hexanoate, isovaleric acid and isoamyl acetate (Ferreira et al. 2000). Quantitatively, various acids formed the most abundant group in the aromatic compounds of these two wines, followed by alcohols and esters. However, ethyl octanoate, ethyl hexanoate, and isoamyl acetate are found to jointly contribute to 97%, 98.9%, and 99% respectively of the global aroma of Cabernet Sauvignon and Cabernet franc wines, which means that fruity notes from ethyl esters are much more important to the contribution of varietal aromatic compounds to the global aroma of the wines (Zhang et al. 2007)

β-Damascenone, a C-13 norisoprenoid compound described previously, has more an indirect than a direct impact on wine aroma in red Bordeaux cultivars. The importance of this aromatic component in both Cabernet Sauvignon and Cabernet franc is due to its capacity for enhanced fruity notes of ethyl cinnamate and caproate and a masking of the herbaceous aroma of isobutyl methoxypyrazines (Pineau et al. 2007).

Alkyl-methoxypyrazines, a family of volatile compounds associated with vegetative/herbaceous characteristics (Allen and Lacey 1998), could be also found in these types of wines (Chapman et al. 2004). However, methoxypyrazines can also be related to undesirable flavour when they exceed concentrations that overpower wine aromas when they are present (Allen and Lacey, 1998). Three important methoxypyrazines have been identified containing a common pyrazine chemical structure: isopropyl methoxypyrazine (IPMP), isobutyl methoxypyrazine (IBMP), and sec-butyl methoxypyrazine (SBMP) (Dunlevy et al. 2009). Each of these compounds is related to a particular aromatic characteristic; e.g. IPMP has a green pepper/asparagus characteristic but in higher concentrations (≈ 0.1-10 μg/L), some earthy, potato or green pea flavours can be detected. IBMP is described as bell pepper/green gooseberry with slight flavor in water, whereas SBMP is described as pea/bell pepper (Bakker and Clarke 2012, Ebeler and Thorngate 2009).

1.7 Literature cited

Acree, T., Barnard, J., and Cunningham, D. (1984). A procedure for the sensory analysis of gas chromatographic effluents. Food Chem. 14:273-286.

Allen, M., and Lacey, M. (1998). Methoxypyrazines of grapes and wines. ACS Symposium Series 714:31-38.

Alleweldt, G., and Dettweiler, E. (1994). The genetic resources of Vitis: world list of grapevine collections, 2nd edn. BAZ IRZ Geilweilerhof, Siebeldingen, Germany.

Alves, R., Nascimento, A., and Nogueira, J. (2005). Characterization of the aroma profile of Madeira wine by sorptive extraction techniques. A. Chim. Acta 546:11-21.

Amati, A. Ferrarini, R. Riponi, C. and Zironi, R. (1983). Una nuova tecnologia per l'appassimento delle uve. Vigne Vini 10:27–35.

Avakyants, S., Rastyannikov, E., Chernyaga, B., and Navrotskii, V. (1981). Khromato-mass-spektrometricheskoe issledovanie letuchikh vesnchestv vina. Vinodel. Vinograd. SSSR 41:50-53.

Bakker, J., and Clarke, R. J. (2012). Wine flavour chemistry (2nd ed.). Chichester, West Sussex; Ames, Iowa: Wiley-Blackwell.

Banerjee, S., and Mazumdar, S. (2012). Electrospray ionization mass spectrometry: A technique to access the information beyond the molecular weight of the analyte. Int. J. Anal. Chem. 2012.

Barata, A., González, S., Malfeito‐Ferreira, M., Querol, A., and Loureiro, V. (2008). Sour rot‐damaged grapes are sources of wine spoilage yeasts. FEMS Yeast Res. 8:1008-1017.

Baumes, R., Wirth, J., Bureau, S., Gunata, Y., and Razungles, A. (2002). Biogeneration of C-13-norisoprenoid compounds: Experiments supportive for an apo-carotenoid pathway in grapevines. *A.* Chim. Acta *458*:3-14.

Bayonove, C., Gunata, Y., Sapis, J., Baumes, R., Dugelay, I., and Grassin, C. (1992). Augmentation des aromes dans le vin et utilisation d'enzymes. Rev Des Oenol Tech Vitivin Oenol 64:15-18.

Bell, A., Ough, C., and Kliewer, W.M. (1979). Effects on must and wine composition, rates of fermentation, and wine quality of nitrogen fertilization of *Vitis vinifera* var. Thompson seedless grapevines. Am. J. Enol. Vitic. 30:124-129.

Bellincontro, A., De Santis, D., Botondi, R., Villa, I., and Mencarelli, F. (2004). Different postharvest dehydration rates affect quality characteristics and volatile compounds of Malvasia, Trebbiano and Sangiovese grapes for wine production. J. Sci. Food Agr. 84:1791-1800.

Bonino, M., Schellino, R., Rizzi, C., Aigotti, R., Delfini, C., and Baiocchi, C. (2003). Aroma compounds of an Italian wine "Ruché" by HS–SPME analysis coupled with GC–ITMS. Food Chem. 80:125-133.

Borneman, A. R., Schmidt, S. A., and Pretorius, I. S. (2012). At the cutting-edge of grape and wine biotechnology. Trends in Genetics. 1:1006-1010.

Boselli, E., Boulton, R.B., Thorngate, J.H., and Frega, N.G. (2004). Chemical and sensory characterization of DOC red wines from Marche (Italy) related to vintage and grape cultivars. J. Agric. Food Chem. 52:3843-3854.

Boulton, R.B. (1980). The relationship between total acidity, titratable acidity and pH in grape tissue. Vitis 19:113-120.

Boursiquot, J.M. and This, P. (1999). Essai de définition du cépage. Prog. Agric. Vitic. 116:359–361.

Bowers, J.E., Bandman, E.B., and Meredith, C.P. (1993). DNA fingerprint characterization of some wine grape cultivars. Am. J. Enol. Vitic. 44:266-274.

Bowers, J.E., and Meredith, C. (1997) The parentage of a classic wine grape, Cabernet Sauvignon. Nat. Genet. 16:84–87.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., and Tabacman, H. (1984). Effect of crop level on growth, yield and wine quality of a high yielding Carignane vineyard. Am. J. Enol. Vitic. 35:247-252.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., and Tabacman, H. (1985). Effect of crop level and crop load on growth, yield, must and wine composition, and quality of Cabernet Sauvignon. Am. J. Enol. Vitic. 36:125-131.

Bureau, S. M., Razungles, A. J., and Baumes, R. L. (2000). The aroma of Muscat of frontignan grapes: Effect of the light environment of vine or bunch on volatiles and glycoconjugates. J. Sci. Food Agr. 80:2012-2020.

Chapman, D. M., Matthews, M. A., and Guinard, J. X. (2004). Sensory attributes of Cabernet Sauvignon wines made from vines with different crop yields. Am. J. Enol. Vitic. 55:325-334.

Chkaiban, L., Botondi, R., Bellincontro, A., Santis, D. d., Kefalas, P., and Mencarelli, F. (2007). Influence of postharvest water stress on lipoxygenase and alcohol dehydrogenase activities, and on the composition of some volatile compounds of Gewürztraminer grapes dehydrated under controlled and uncontrolled thermohygrometric conditions. Austral. J. Grape and Wine Res. 13:142-149.

Comuzzo, P., Tat, L., Tonizzo, A., and Battistutta, F. (2006). Yeast derivatives (extracts and autolysates) in winemaking: Release of volatile compounds and effects on wine aroma volatility. Food Chem. 99:217-230.

Constantini, V., Bellincontro, A., De Santis, D., Botondi, R., and Mencarelli, F. (2006). Metabolic changes of Malvasia grapes for wine production during postharvest drying. J. Agr. Food Chem. 54:3334-3340.

Corte, V., Oliva, D., Ragusa, M., Genna, G., Strano, M. and Di Stefano, R. (2001). Aspetti tecnici, microbiologici e chimici connessi con i sistemi di appassimento delle uve. Enologo 12:87–97.

Cox, A., Capone, D. L., Elsey, G. M., Perkins, M. V., and Sefton, M. A. (2005a). Quantitative analysis, occurrence, and stability of (E)-1-(2, 3, 6-trimethylphenyl) buta-1, 3-diene in wine. J. Agri. Food Chem. 53:3584-3591.

Cox, A., Skouroumounis, G. K., Elsey, G. M., Perkins, M. V., and Sefton, M. A. (2005b). Generation of (E)-1-(2,3,6-trimethylphenyl) buta-1,3-diene from C13-norisoprenoid precursors. J. Agri. Food Chem. 53:6777-6783.

Cullère, L., Escudero, A., Cacho, J., and Ferreira, V. (2004) Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. J. Agric. Food. Chem. 52:1653-1660.

Delcroix, A., Günata, Z., Sapis, J., Salmon, J., and Bayonove, C. (1994). Glycosidase activities of three enological yeast strains during winemaking: Effect on the terpenol content of muscat wine. Am. J. Enol. Vitic*. 45*:291-296.

Demyttenaere, J. C., Sánchez-Martinez, J. I., Verhe, R., Sandra, P. and De Kimpe, N. (2003). Analysis of volatiles of malt whisky by solid-phase microextraction and stir bar sorptive extraction. J. Chrom. A. 985:221-232.

Di Profio, F., Reynolds, A.G., and Kasimos, A. (2011a). Canopy management and enzyme impacts on Merlot, Cabernet franc, and Cabernet Sauvignon. I. Yield and berry composition. Am. J. Enol. Vitic. 62:139-151.

Di Profio, F., Reynolds, A. G., and Kasimos, A. (2011b). Canopy management and enzyme impacts on Merlot, Cabernet franc, and Cabernet Sauvignon. II. Wine composition and quality. Am. J. Enol. Vitic. 62:152-168.

Dimitriadis, E., and Williams, P. (1984). The development and use of a rapid analytical technique for estimation of free and potentially volatile monoterpene flavorants of grapes. Am. J. Enol. Vitic. 35:66-71.

Donèche, B.J. (1993). Botrytized wines. *In* Wine Microbiology and Biotechnology, Ed. Fleet, pp. 327-346. Harwood Academic Publisher, Chur, Switzerland.

Douglas, D., Cliff, M. A., and Reynolds, A. G. (2001). Canadian terroir: Characterization of Riesling wines from the Niagara Peninsula. Food Res. Int. *34*:559-563.

Dunlevy, J., Kalua, C., Keyzers, R., and Boss, P. (2009). The production of flavour & aroma compounds in grape berries. Grapevine Mol. Phy. Biot. 293-340.

Ebeler, S. E., and Thorngate, J. H. (2009). Wine chemistry and flavor: Looking into the crystal glass. J. Agri. Food Chem. 57:8098-8108.

Edson, C. G., Howell, G. S., and Flore, J. (1993). Influence of crop load on photosynthesis and dry matter partitioning of Seyval grapevines I. single leaf and whole vine response pre-and post-harvest. Am. J. Enol. Vitic. 44:139-147.

Edson, C. G., Howell, G. S., and Flore, J. (1995). Influence of crop load on photosynthesis and dry matter partitioning of seyval grapevines. III. Seasonal changes in dry matter partitioning, vine morphology, yield, and fruit composition. Am. J. Enol. Vitic. 46:478-485.

Escudero, A., Gogorza, B., Melus, M., Ortin, N., Cacho, J., and Ferreira, V. (2004). Characterization of the aroma of a wine from maccabeo. key role played by compounds with low odor activity values. J. Agric. F. Chem. 52:3516-3524.

Escudero, A., Campo, E., Fariña, L., Cacho, J., and Ferreira, V. (2007). Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. J. Agri. Food Chem. 55:4501-4510.

Fang, F., Li, J., Zhang, P., Tang, K., Wang, W., Pan, Q., and Huang, W. (2008). Effects of grape variety, harvest date, fermentation vessel and wine ageing on flavonoid concentration in red wines. Food Res. Int. 41:53-60.

Ferreira, V., and Cacho, J. (2009). Identification of impact odorants of wines. Wine Chem. Biochem. 393-415.

Ferreira, V., López, R., and Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. J. Sci. Food Agri., 80:1659-1667.

Fischer, U., Roth, D., and Christmann, M. (1999). The impact of geographic origin, vintage and wine estate on sensory properties of *Vitis vinifera* cv. Riesling wines. Food Qual. Pref. 10:281-288.

Fisher, K.H., Bradt, O., Wiebe, J., and Dirks, V. (1977). Cluster-thinning 'De Chaunac' French hybrid grapes improves vine vigor and fruit quality in Ontario. J. Am. Soc. Hort. Sci. 102:162-165.

Fleet, G.H. (1993). Wine: Microbiology and Biotechnology. CRC Press.

Francis, I., and Newton, J. (2008). Determining wine aroma from compositional data. Aus. J. Grape and Wine Res., 11:114-126.

Freeman, B.M. and Kliewer, M. (1983). Effect of irrigation, crop level and potassium fertilization on Carignane vines. II. Grape and wine quality. Am. J. Enol. Vitic. 34:197-207.

Fuleki, T. and Francis, F. J. (1968). Quantitative methods for anthocyanins. J. Food Sci. 33:266-274.

García-Romero, E., Pérez-Coello, M., Cabezudo, M., Sánchez-Muñoz, G., and Martín-Alvarez, P. (1999). Fruity flavor increase of Spanish Airén white wines made by brief fermentation skin contact/Aumento del aroma afrutado de los vinos blancos airén fermentados en presencia de hollejos. Food Sci. Tech. Int. 5:149-157.

Grosch, W. (1993). Detection of potent odorants in foods by aroma extract dilution analysis. T. Food Sci. Tech. 4:68-73.

Guerzoni, E., and Marchetti, R. (1987). Analysis of yeast flora associated with grape sour rot and of the chemical disease markers. Appl. Env. Microbiol. 53:571-576.

Günata, Y. Z., Bayonove, C. L., Baumes, R. L. and Cordonnier, R.E. (1985) The aroma of grapes. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. J Chrom. A. 331:83-90.

Guth, H. (1997). Identification of character impact odorants of different white wine varieties. J. Agri. Food Chem. 45:3022-3026.

Hale, C. (1977). Relation between potassium and the malate and tartrate contents of grape berries. Vitis 16:9-19.

Hardy, P. (1970). Changes in volatiles of muscat grapes during ripening. Phytochemistry 9:709-715.

Harris, D. (2001). Análisis químico cuantitativo.2ª ed. Ed.Reverte, México.

Hashizume, K. (1999). Grape maturity and light exposure affect berry methoxypyrazine concentration. Am. J. Enol. Vitic. 50:194.

Hashizume, K., Tozawa, K., Endo, M., and Aramaki, I. (2001). S-adenosyl-L-methionine-dependent O-methylation of 2-hydroxy-3-alkylpyrazine in wine grapes: A putative final step of methoxypyrazine biosynthesis. Bios. Biotech. Biochem. 65:795-801.

Hayasaka, Y., MacNamara, K., Baldock, G. A., Taylor, R. L., and Pollnitz, A. P. (2003). Application of stir bar sorptive extraction for wine analysis. A. Bioa. Chem. 375:948-955.

Hepner, Y. and Bravdo B. (1985). Effect of crop level and drip irrigation scheduling on the potassium status of Cabernet Sauvignon and Carignane vines and its influence on must and wine composition and quality. Am. J. Enol. Vitic. 36:140-147.

Herbert, P., Cabrita, M. J., Ratola, N., Laureano, O., and Alves, A. (2005). Free amino acids and biogenic amines in wines and musts from the Alentejo region. evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage. J. Food Eng. 66:315-322.

Heyworth, R. and Walker, P. (1962). Almond-emulsin β-d-glucosidase and β-d-galactosidase. Biochem. J. 83:331-335.

Himelrick, D. G. (2003). Handling, storage and postharvest physiology of Muscadine grapes: A review. Small Fruit Reviews 2:45-62.

Hocquigny, S., Pelsy, F., Dumas, V., Kindt, S., Heloir, M., and Merdinoglu, D. (2004). Diversification within grapevine cultivars goes through chimeric states. Genome. 47:579-589.

Howard, K. L., Mike, J. H., and Riesen, R. (2005). Validation of a solid-phase microextraction method for headspace analysis of wine aroma components. Am. J. Enol. Vitic. 56:37-45.

Jackson, D., and Lombard, P. (1993). Environmental and management practices affecting grape composition and wine quality-a review. Am. J. Enol. Vitic. 44:409-430.

Jackson, D., and Schuster, D. (2001). The Production of Grapes and Wine in Cool Climates. Daphne Brasell Associates Ltd and Gypsum Press, Aotearoa, New Zealand.

Jones, G. V., and Davis, R. E. (2000). Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. Am. J. Enol. Vitic. 51:249-261.

Kataoka, I., Kubo, Y., Sugiura, A., and Tomana, T. (1984). Effects of temperature, cluster shading and some growth regulators on L-phenylalanine ammonia-lyase activity and anthocyanin accumulation in black grapes. Memoirs of the College of Agriculture-Kyoto University. 124:35-72

Kays, S.J. (1997). Stress in harvested products, *in* Postharvest Physiology in Perishable Plant Products, Ed by Kays, S. J. pp 335–408. Exon Press. Athens, GA..

Keller, M., Mills, L. J., Wample, R. L., and Spayd, S. E. (2005). Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. Am. J. Enol. Vitic. 56:91-103.

Kennedy, J. (2002). Understanding grape berry development. Practical Winery & Vineyard. San Rafael, California.

Kita, M., Hirata, Y., Moriguchi, T., Endo-Inagaki, T., Matsumoto, R., Hasegawa, S., Suhayda, C.G., and Omura, M. (2000). Molecular cloning and characterization of a novel gene encoding limonoid UDP-glucosyltransferase in Citrus. FEBS Letters. 469:173–178.

Kliewer, W. M. (1971). Effect of day temperature and light intensity on concentration of malic and tartaric acids in Vitis vinifera L. grapes. J. Am. Soc. Hort. Sci. 96:372-377.

Kliewer, W. M. (1977). Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. Am. J. Enol. Vitic. 28:96-103.

Kliewer, W. M., and Dokoozlian, N.K. (2005). Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. Am. J. Enol. Vitic. 56:170-181.

Kliewer, W. M., Howarth, L., and Omori, M. (1967). Concentrations of tartaric acid and malic acids and their salts in *Vitis vinifera* grapes. Am. J. Enol. Vitic. 18:42-54.

Kliewer, W. M., Marois, J., Bledsoe, A., Smith, S., Benz, M., and Silvestroni, O. (1988). Relative effectiveness of leaf removal, shoot positioning, and trellising for improving winegrape composition. Proceedings of the Second International Symposium for Cool Climate Viticulture and Oenology. RE Smart et al.(Eds.), 123-126.

Kliewer, W. M., and Ough, C. (1970). The effect of leaf area and crop level on the concentration of amino acids and total nitrogen in Thompson Seedless grapes. Vitis 9:196-206.

Kliewer, W. M., and Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. Am. J. Enol. Vitic. 23:71-77.

Kliewer, W. M., and Weaver, R. (1971). Effect of crop level and leaf area on growth, composition, and coloration of Tokay grapes. Am. J. Enol. Vitic. 22:172-177.

Langenzersdorf, G. (2000). Differentiation and identification of White Riesling clones by genetic markers. Vitis. 39:103-107.

Leclerc, M., Arnaud, A., Ratomahenina, R., and Galzy, P. (1987). Yeast ß-glucosidases. Biotech. Gen. Eng. Rev. 5:269-296.

Lee, J., Hwang, G., Van Den Berg, F., Lee, C., and Hong, Y. (2009). Evidence of vintage effects on grape wines using NMR-based metabolomic study. Anal. Chim. Act. 648:71-76.

López, R., Ortín, N., Pérez-Trujillo, J. P., Cacho, J., and Ferreira, V. (2003). Impact odorants of different young white wines from the Canary Islands. J. Agric. Food Chem. 51:3419-3425.

Macaulay, L., and Morris, J. (1993). Influence of cluster exposure and winemaking processes on monoterpenes and wine olfactory evaluation of Golden Muscat. Am. J. Enol. Vitic. 44:198-204.

Marais, J. (1983). Terpenes in the aroma of grapes and wines: A review. S. Afr. J. Enol. Vitic. 4:49-60.

Marais, J., Van Wyk, C., and Rapp, A. (1992). Effect of sunlight and shade on norisoprenoid levels in maturing Weisser Riesling and Chenin blanc grapes and Weisser Riesling wines. S. Afr. J. Enol. Vitic. 13:23-32.

Mateo, J., and Jiménez, M. (2000). Monoterpenes in grape juice and wines. J. Chrom. A. 881:557-567.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., and Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. J. Agr. Food Chem. 10:4009-4017.

McCarthy, M. (1999). Weight loss from ripening berries of Shiraz grapevines (*Vitis vinifera* L. cv. Shiraz). Austral. J. Grape Wine Res. 5:10-16.

Mendes-Pinto, M. M. (2009). Carotenoid breakdown products. The norisoprenoids in wine aroma. Arch. Biochem. Biophy. 483:236-245.

Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. Food Res. Int. 43:1844-1855.

Molitor, D., Behr, M., Hoffmann, L., and Evers, D. (2012). Impact of grape cluster division on cluster morphology and bunch rot epidemic. Am. J. Enol. Vitic. 63:508-514.

Moreno, J. J., Cerpa-Calderón, F., Cohen, S. D., Fang, Y., Qian, M., and Kennedy, J. A. (2008). Effect of postharvest dehydration on the composition of Pinot noir grapes "*Vitis vinifera*" L. and wine. Food Chem. 109:755-762.

Morris, J., and Cawthon, D. (1982). Effect of irrigation, fruit load, and potassium fertilization on yield, quality, and petiole analysis of Concord (*Vitis labrusca* L.) grapes. Am. J. Enol. Vitic. 33:145-148.

Mortensen, J. (1984). Effect of bunch and grape maturity on finished Muscadine wine. Ratio. 28:22.

Mullins, M. G., Bouquet, A., & Williams, L. E. (1992). Biology of the grapevine Cambridge University Press.

Nelson, R. R., and Acree, T. E. (1978). Concord wine composition as affected by maturity and processing technique. Am. J. Enol. Vitic. 29:83-86.

Nongonierma, A., Voilley, A., Cayot, P., Le Quéré, J. L., and Springett, M. (2006). Mechanisms of extraction of aroma compounds from foods, using adsorbants. Effect of various parameters. Food Rev. Int. 22:51-94.

Nykanen, L. (1986) Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. Am. J. Enol. Vitic. 37:84-96.

Oliveira, C., Silva Ferreira, A.C., Mendes Pinto, M., Hogg, T., Alves, F. and Guedes De Pinho, P. (2003). Carotenoid compounds in grapes and their relationship to plant water status. J. Agr. Food Chem. 51:5967–5971.

OMAFRA, Ontario Ministry of Agriculture, Food and Rural Affairs. 2009a. Botrytis. Queen´s Printer, Ontario, 1-6 pp. (www.omafra.gov.on.ca/IPM/english/grapes/diseases-and-disorders/botrytis.html) acces octuber 10, 2012.

OMAFRA, Ontario Ministry of Agriculture, Food and Rural Affairs. 2009b Grapevine leafroll virus. Queen´s Printer, Ontario, 1-8 pp. (www.omafra.gov.on.ca/IPM/english/grapes/diseases-and-disorders/leafroll.html) acces octuber 10, 2012.

Ortega-Heras, M. González-SanJose, M. L. and Beltran, S. (2002). Aroma composition of wine studied by different extraction methods. Anal. Chim. Act. 458:85-93.

Ough, C., and Nagaoka, R. (1984). Effect of cluster thinning and vineyard yields on grape and wine composition and wine quality of Cabernet Sauvignon. Am. J. Enol. Vitic. 35:30-34.

Palomo, E. S., Pérez-Coello, M., Díaz-Maroto, M., González Viñas, M., and Cabezudo, M. (2006). Contribution of free and glycosidically-bound volatile compounds to the aroma of Muscat á Petit grains wines and effect of skin contact. Food Chem. 95:279-289.

Pelsy, F. (2009). Molecular and cellular mechanisms of diversity within grapevine varieties. Heredity 104:331-340.

Pelsy, F., Hocquigny, S., Moncada, X., Barbeau, G., Forget, D., Hinrichsen, P., and Merdinoglu, D. (2010). An extensive study of the genetic diversity within seven French wine grape variety collections. Theor. Appl. Gen. 120:1219-1231.

Pereira, G.E., Gaudillere, J., van Leeuwen, C., Hilbert, G., Maucourt, M., Deborde, C., and Rolin, D. (2006). H-NMR metabolite fingerprints of grape berry: Comparison of vintage and soil effects in Bordeaux grapevine growing areas. Anal. Chim. Acta. 563:346-352.

Pérez-Magariño, S., and González-San José, M. L. (2004). Evolution of flavanols, anthocyanins, and their derivatives during the aging of red wines elaborated from grapes harvested at different stages of ripening. J. Agr. Food Chem. 52:1181-1189

Pigott, S. (1991). Riesling. Viking Press, London, UK.

Pineau, B., Barbe, J., van Leeuwen, C., and Dubourdieu, D. (2007). Impact for β -damascenone on red wines aroma J. Agr. Food Chem. 55:4103-4108.

Polaskova, P., Herszage, J., and Ebeler, S.E. (2008). Wine flavor: Chemistry in a glass. Chem. S. Rev. 37:2478-2489

Rankine, B., Fornachon, J., Boehm, E., and Cellier, K. (1971). Influence of grape variety, climate and soil on grape composition and on the composition and quality of table wines. Vitis. 10:33-50.

Rapp, A., and Mandery, H. (1986). Wine aroma. Experientia. *42*:873-884.

Rapp, A. (1998). Volatile flavor of wine: Correlation between instrumental analysis and sensory perception. Nahrung. 2:351-363.

Razungles, A., Babic, I., Sapis, J. and Bayonove, C. (1996) Particular behaviour of epoxy xanthophylls during véraison and maturation of grapes. J. Agr. Food Chem. 44:3821–3825.

Reineccius, G. (2005). Flavor chemistry and technology 2nd Ed. CRC press. Boca Raton, FL.

Regner, F., Stadlbauer, A., Eisenheld, C., and Kaserer, H. (2000). Genetic relationships among Pinots and related cultivars. Am. J. Enol. Vitic. 51:7-14.

Reynolds, A. G., Parchomchuk, P., Berard, R., Naylor, A. P., and Hogue, E. (2006). Gewürztraminer grapevines respond to length of water stress duration. Int. J. Fruit Sci. 5:75-94.

Reynolds, A. G., Pool, R. M., and Mattick, L. R. (1986a). Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. Vitis 25:85-95.

Reynolds, A. G., Pool, R. M., and Mattick, L. R. (1986b). Effect of shoot density and crop control on growth, yield, fruit composition, and wine quality of Seyval blanc grapes. J. Am. Soc. Hortic. Sci. 111:55-63.

Reynolds, A. G., Price, S. F., Wardle D. A., and Watson, B.T. (1994). Fruit environment and crop level effects on Pinot noir. I. vine performance and fruit composition in British Columbia. Am. J. Enol. Vitic. 45:452-459.

Reynolds, A. G., Schlosser, J., Sorokowsky, D., Roberts, R., Willwerth, J., and de Savigny, C. (2007). Magnitude of viticultural and enological effects. II. relative impacts of cluster thinning and yeast strain on composition and sensory attributes of chardonnay musqué. Am. J. Enol. Vitic*. 58*:25-41.

Reynolds, A. G., and Wardle, D .A. (1989). Impact of various canopy manipulation techniques on growth, yield, fruit composition, and wine quality of Gewürztraminer. Am. J. Enol. Vitic. 40:121-129.

Reynolds, A. G., Yerle, S., Watson, B. T., Price, S. F., and Wardle, D. A. (1996). Fruit environment and crop level effects on Pinot noir. III. composition and descriptive analysis of Oregon and British Columbia wines. Am. J. Enol. Vitic. 47:329-339.

Robinson, J. (1996). Jancis Robinson's guide to wine grapes. Oxford University Press.

Rubiolo, P., Sgorbini, B., Liberto, E., Cordero, C., and Bicchi, C. (2010). Analysis of the plant volatile fraction. *In* The Chemistry and Biology of Volatiles. A. Herrmann, Ed, pp, 49-93. Wiley and Son Publications. West Sussex, UK.

Rusjan, D. (2010). Aromas in grape and wine. Methodologies and results in grapevine research pp. 411-442.

Sarry, J., and Günata, Z. (2004). Plant and microbial glycoside hydrolases: Volatile release from glycosidic aroma precursors. Food Chem. 87:509-52.

Shaulis, N. J. (1980). Responses of grapevines and grapes to spacing of and within canopies. Grape and Wine Centennial Symposium Proceedings, A. D. Webb (Ed.) pp. 18-21. Univ. of Calif. Davis. CA.

Smart, R. E., Robinson, J., Due, G., and Brien, C. (1985a). Canopy microclimate modification for the cultivar Shiraz. I. Definition of canopy microclimate. Vitis 24:17-31.

Smart, R. E., Robinson, J., Due, G., and Brien, C. (1985b). Canopy microclimate modification for the cultivar Shiraz. II. Effects on must and wine composition. Vitis 24:119-128.

Smart, R., Turkington, C., and Evans, J. (1974). Grapevine response to furrow and trickle irrigation. Am. J. Enol. Vitic. 25:62-66.

Somers, T. (1975). In search of quality for red wines. Food Tech. Austral. 27:49-56.

Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. Am. J. Enol. Vitic. 53:171-182.

Sponholz, W. (1993). Wine spoilage by microorganisms. *In* Wine Microbiology and Biotechnology, Fleet, G. H. (Ed), pp. 395-420. Harwood Academic Publisher, Chur, Switzerland.

This, P., Lacombe, T., and Thomas, M. R. (2006). Historical origins and genetic diversity of wine grapes. Trends in Genetics 22:511-519.

Truel P, Rennes C, Domergue P (1980) Identification in collections of grapevines. In: Third international symposium on grape breeding. Department of Viticulture and Enology University of California, Davis, CA, pp 78–86.

Tominaga, T., Baltenweck-Guyot, R., Peyrot des Gachons, C. P., and Dubourdieu, D. (2000). Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. Am. J. Enol. Vitic. 51:178-181.

Vasconcelos, M. C. and Castagnoli, S. (2000). Leaf canopy structure and vine performance. Am. J. Enol. Vitic. 51:390-396.

Vilanova, M. and Sieiro, C. (2006). Determination of free and bound terpene compounds in Albariño wine. J. Food Comp. An. 19:694-697.

Vasserot, Y., Caillet, S., and Maujean, A. (1997). Study of anthocyanin adsorption by yeast lees. Effect of some physicochemical parameters. Am. J. Enol. Vitic. 48:433-437.

Wang, N., and Brennan, J. (1995). Changes in structure, density and porosity of potato during dehydration. J. Food Eng. 24:61-76.

Weaver, R. J., and McCune, S. B. (1960). Effects of overcropping Alicante Bouschet grapevines in relation to carbohydrate nutrition and development of the vine. Proc. Am. Soc. Hortic. Sci. 75:341-353.

Weaver, R. J., McCune, S. B., and Amerine, M.A. (1961). Effect of level of crop on vine behavior and wine composition in Carignane and Grenache grapes. Am. J. Enol. Vitic. 12:175-184.

Weaver, R. J., and Pool, R. M. (1968). Effect of various levels of cropping on *Vitis vinifera* grapevines. Am. J. Enol. Vitic. 19:185-193.

Winkler, A. J. (1954). Effects of overcropping. Am. J. Enol. Vitic. 5:4-12.

Winkler, A. J., and Williams, W. (1940). The heat required to bring Tokay grapes to maturity. Proc. Am. Soc. Hortic. Sci. 37:650-652.

Wolf, T., Miller, K., LoGiudice, D., Low, C., Engleman, G., and Beanland, L. (2002) Cabernet franc: Viticultural aspects. *In* ASEV/ES 27th annual meeting. Virginia Tech.

Wolpert, J., Kasimatis, A., and Verdegaal, P. (1995). Viticultural performance of seven cabernet sauvignon clones in the northern San Joaquin Valley, California. Am. J. Enol. Vitic. *46*:437-441.

Zalacain, A., Marín, J., Alonso, G., and Salinas, M. (2007). Analysis of wine primary aroma compounds by stir bar sorptive extraction. Talanta. 71:1610-1615.

Zamboni, A., Minoia, L., Ferrarini, A., Tornielli, G. B., Zago, E., Delledonne, M., and Pezzotti, M. (2008). Molecular analysis of post-harvest withering in grape by AFLP transcriptional profiling. J. Exp. Bot. 59:4145-4159

Zhang, M., Xu, Q., Duan, C., Qu, W., and Wu, Y. (2007). Comparative study of aromatic compounds in young red wines from Cabernet Sauvignon, Cabernet franc, and Cabernet Gernischet varieties in China. J. Food Sci. 72:C248-C252.

Zoecklein, B., Williams, J., and Duncan, S. (2010). Effect of sour rot on the composition of White Riesling (*Vitis vinifera* L.) grapes. Small Fruit Reviews 1:63-77.

CHAPTER 2

IMPACT OF CROP LEVEL AND HANG TIME ON THE COMPOSITION OF FOUR WINE GRAPE CULTIVARS FROM THE NIAGARA REGION

Luis H. Moreno Luna and Andrew G. Reynolds

2.1 Abstract: Pinot gris, Riesling, Cabernet franc and Cabernet Sauvignon vines from a single vineyard in Niagara-on-the-lake, Ontario, Canada, were subjected to two viticultural treatments during 2011 and 2012 vintages in a randomized experiment. Two crop levels included full crop (FC) and half crop (HC), whereby crop was reduced to one basal cluster per shoot at veraison. Crop level treatments were combined with three harvest dates: T0 (commercial harvest), T1 (three weeks after T0), T2 (six weeks after T0), all with subsequent wine production. It was decided to experiment with increased hang time to determine whether keeping a full crop with a longer hang time might have a greater impact on wine quality than to reduce the crop level. Analysis of juice, must and wine were carried out and results statistically analyzed. In general, reductions in crop led to an increase in Brix, reduced yield and clusters per vine in all cultivars, and an increase in cluster weight in Cabernet franc. Increased hang time also led to an increase in Brix and pH, and reductions in titratable acidity and berry weight. Effect of hang time in berries was extrapolated to must and consecutively to the wine; increase in pH and acidity had minimal effect on the overall quality other than reduction in phenols and color in red cultivars. The effect of vintage was important since an increase in Brix and yields were greater in 2012, a dry-cooler year during harvests than 2011 rainy-warmer, with a better performance in overall chemistry in wines.

Key words: Crop reduction, hang. time, vintage effect, harvest delay, grape composition, wine quality

2.2 Introduction

The use of crop reduction is a practice that is followed by wine producers, and it is an important factor that could affect the interaction between vine morphological development and physiological response (Edson et al. 1995). Manipulation of crop could affect the characteristics in final wine due to changes in fruit composition. Vines with higher crop loads allocate their carbon resource to fruit production with a reduction in shoot growth, leaf size and leaf area (Bravdo et al. 1984; Edson et al. 1993).

The effects of crop level reduction are typically an increase in Brix, anthocyanins, total phenols, and color intensity (Jackson and Lombard 1993; Mazza et al. 1999; Reynolds et al. 1994). Increases in fruit quality and yield go concomitantly with vine capacity due to regulation of pruning severity and fruit thinning (Weaver et al. 1961) and increase of berry weight (Freeman and Kliewer 1983).

The use of hang time manipulation is partially linked to the reduction of berry weight related to loss of water resulting in increase in sugar concentration (Bellincontro et al. 2004; Moreno et al. 2008). The first premise of the effect of hang time is that grapes that remain on the vines beyond the normal time to attain a higher level of sugar become physically overmature. Such grapes are more susceptible to handling and transportation injury (Winkler 1954). In the case of wine grapes, this process is generated by dehydration and sometimes promoted to increase sugar content by concentration (Constantini et al. 2006).

It was decided to experiment with different “hang times” (harvest dates) to determine whether keeping a full crop with a more lengthy hang time might have a greater impact on wine quality than to reduce the crop level during veraison. The overall objective for this project was to determine the impact of hang time and crop control on the composition of four grape cultivars: two whites (Riesling and Pinot gris), and two reds (Cabernet Sauvignon and Cabernet franc) commonly produced in the Niagara Peninsula.

2.3 Materials and Methods

2.3.1 Treatment set up

All trials were carried out at Pondview Estate Winery within the Four Mile Creek, VQA *sub-appellation* in the Niagara Peninsula. This *sub-appellation* is located in the central plain in Niagara-on-the-Lake, slightly inland from Lake Ontario and below the bench of the Niagara Escarpment (VQA Ontario Four Mile Creek, 2013). The region is characterized by a mean temperature in July of 22.6 ºC with precipitation of 541 mm during the growing season (Vine and Tree Fruit INnovations, 2013). Soil characteristics are red shale parent material with high silt and clay content, mostly Halton till, with a mix of Chinguacousy, Peel, Jeedo soils with a high water holding capacity and an average elevation of 95 m above sea level (Haynes 2000; Kingston and Presant 1989).

Two crop level treatments were imposed at veraison in both years:

Full crop: For this treatment all clusters were retained on every shoot with subsequent harvest.

Half crop: For this treatment only one cluster per shoot (basal cluster) was retained

Each cultivar (P. Gris, Riesling, C. Franc and C. Sauvignon) was divided into blocks, where each block was comprised of six rows containing six, six-vine panels. Experimental design was a randomized complete block with a factorialized treatment arrangement. A total of 216 vines per cultivar and 864 vines in total were used for the experiment.

2.3.2 Harvest period

The dates of harvest are shown in Table 2.3 for 2011 and 2012 respectively. Manual harvest was employed for all treatments and was divided into three hang times as follows:

- **First harvest (T0):** The date of harvest was the same as the regular date harvest of the vineyard indicated by the owner (regular harvest).
- **Second harvest (T1):** 3 weeks after regular harvest.

- **Third harvest (T2):** 6 weeks after regular harvest.

2.3.3 Vine size and Crop load

All vines were pruned during the middle of March in 2011 and 2012. Vines were cane pruned at the same number of nodes on each cultivar with an average of 30 nodes with three canes per vine. Pruning weights per vine (vine size) were measured *in situ* with an Oster dairy scale (Model 80:50; Jarden Corp., Rye, NY). Vine size was reported as kg/vine and was used to calculate crop load (Ravaz 1903) by dividing the yield per vine/weight of cane pruning per vine.

2.3.4 Sampling

Two samples of 150 berries per sample were collected during every harvest time (T0, T1, T2) from every panel (6 x 2 x crop level) with a total of 24 samples per block. Each sample was stored in identified plastic bags at -25 ºC until analysis. All fruit from each vine were weighed *in situ* by an electronic scale and recorded. Fruit in each panel was consolidated and transported in plastic trays to the experimental pilot winery at CCOVI, Brock University for further processing. For wine analysis, samples were collected directly from final wine and kept at 4 ºC. After each sampling N_2 inert gas was supplied at the headspace of the wines to avoid oxidation.

2.3.5 Crushing and pressing

All fruit from the white cultivars was de-stemmed and crushed using a stainless steel motorized crusher-destemmer (Model Pillan N1, Enoitalia., San Miniato Italy) and pressed same day as harvest with a 40-L bladder water-press (Model LDC5518, Enoitalia., San Miniato, Italy) at 2 bars upon dryness. A 250-mL must sample was collected from each treatment replicate and stored at -25 ºC for further analysis. Must was collected in Vitromex glass carboys and stored at 4 ºC with addition of 50 ppm of sulfur dioxide (SO_2). Must was racked 24 hours after and placed in a fermentation room overnight prior to inoculation.

Fruit from red cultivars was crushed and de-stemmed using a stainless steel motorized crusher-destemmer, fermented on skins at 25 ºC (see 2.3.6 Fermentation) with punch down every 12 hours until end of fermentation, and then pressed upon dryness at 2 bar with 40-L water bladder water-press. Must

samples were collected from each treatment replicate prior to inoculation as with white cultivars. Wine was thereafter collected in Vitromex glass carboys and stored at 25 ºC overnight for settling. Racking was carried out before malolactic fermentation.

2.3.6 Fermentation and bottling

Commercial *S. cerevisiae* (ex-*bayanus*) E1118 Lalvin (Lallemand, St Simon, France) was used for alcoholic fermentation at 0.25 g/L for white cultivars in an 18 ºC fermentation chamber, and 0.3 g/L for red cultivars in a 25 ºC fermentation chamber. All cultivars were fermented to dryness. The yeast was rehydrated at 38 ºC in 10 times its weight in water for 20 minutes followed by addition of 10 times its weight with must at room temperature for 15 minutes. This final mix was directly added to must. Diammonium phosphate (DAP) as a nitrogen source was added once at 0.4 g/L. Brix was measured daily to follow fermentation using a Brix hydrometer in 500 mL of must free of suspended solids. Once fermentation reached zero Brix, a 20-mL sample was measured in a FOSS Wine Scan FT120 (Model 77310 FOSS Electric, Hillerø, Denmark) to determine residual sugars with commercial calibration. Fermentation was considered finished when residual sugar in the wine samples was zero.

For red cultivars, after alcoholic fermentation, pressing and racking, malolactic fermentation (MLF) was carried out with the addition of 0.25 g/L of *Oenococcus oeni* VP41 (Lallemand, St. Simon, France) in a 25 ºC fermentation chamber. MLF was stopped with 50 ppm of SO_2 once the concentration of malic acid was equal or close to zero as obtained through a FOSS Wine Scan FT120.

Final wines were racked and stored in Vitromex glass carboys at 4 ºC with 50 ppm of free SO_2 until bottling. Free SO_2 analysis was done periodically to ensure 50 ppm concentration during storage. All wines from 2011 and 2012 were bottled at room temperature in presence of N_2 inert gas to avoid oxidation. Wine was passed through a plate and frame filter (Scott Laboratories, Pickering, ON) using 8 in x 8 in, 3.5 mm pad filters (Scott Laboratories, Pickering, ON) and 0.45 μ membrane filter (Millipore, Bedford, MA) and then bottled in 750 mL bottles. All bottled wine was stored in the CCOVI cellar at 12º C.

2.3.7 Analysis of berries

Berry samples for white and red cultivars were removed from -25 ºC freezer, weighed and defrosted in an 80 ºC water bath for 1 hour and then processed with an Omega Juicer (Model 500, Omega products Inc., Ft. Lauderdale, Florida, USA). Juice was analyzed for pH with standardized electrode in an AR50 pH meter (Model AR93312527, Fisher Scientific, Singapore). Brix was obtained with an Abbé refractometer (Model 10450, American Optical, Buffalo, NY,). Titratable acidity (TA) was obtained after centrifugation of 25 mL of juice at 4500 rpm for 10 minutes in an IEC centrifuge (Model CL2, Thermo Scientific, Waltham, MA), with an automatic PC-titrate (Model PC1300-475, Man-Tech Associated Inc., Guelph, ON) to a pH end point pH 8.0, using 0.1 N sodium hydroxide.

Total anthocyanin concentration was determined in duplicate for red cultivars using a modified version of the Fuleki and Francis (1968) pH shift method using pH 1.0 and pH 4.5 buffer solutions prepared with 0.2M KCl with 0.2M HCl and 1M sodium acetate with 1M HCl respectively. A sample of 1 mL of centrifuged juice was diluted in 9 mL of distilled water in screw-top test tub and mixed in Vortex (Thermolyne, Dubuque, Iowa). One sample of 0.1 mL was mixed in Vortex with 1.9 mL placed in 10-mL light-visible cuvettes of each buffer and held in the dark for one hr. Subsequently absorbance was measured at 520 nm in an UV/Vis spectrometer (Biochrom Ltd., Cambridge, England). The total anthocyanin concentration was calculated with the formula: total anthocyanin (mg/L) = [A_{520} (pH 1.0 – pH 4.5) /0.0042] x [dilution factor] and expressed as malvidin equivalents.

Color intensity and hue in red cultivars were calculated from a modified method proposed by Mazza et al. (1999). A pH 3.5 buffer solution was prepared from 0.1M citric acid and 0.2M Na_2HPO_4. A sample of 0.1 mL was obtained from the previous 10 mL mix used for anthocyanins described above and mixed with 1.9 mL of buffer. Absorbance values were measured at 420 nm and 520 nm for each cuvette where the color intensity = $A_{420} + A_{520}$, and the hue = A_{420}/A_{520}.

Total phenol concentration in red cultivars was obtained following the Folin-Ciocalteu micro method for total phenols (Singleton and Rossi 1965; Waterhouse 2006). A sample of 1 mL of juice, prior centrifuged and filtered in a 10 μm filter (Millipore, Bedford, MA., USA) , was diluted with 9 mL of distilled water; a sample of 20 μL from this mix was pipetted into light-visible cuvette followed by 1.58 mL of water, 100 μL of 2N Folin-Ciocalteu phenol reagent (Sigma-Aldrich, 77310) and mixed in Vortex. After 8 min, 300 μL of sodium carbonate solution was added, shaken to mix and held in dark during 2 hrs. at 20ºC. Determination of absorbance at 765 nm was used to calculate concentration of gallic acid equivalents extrapolating each absorbance into a calibration curve of gallic acid previously prepared (See Appendix Figure 2.2).

2.3.8 Analysis of must

Samples of must stored in plastic bottles were defrosted in an 80 ºC water bath for 1 hr. Analysis of pH, TA and Brix of must, previously centrifuged and filtered, was carried out in white cultivars, as described in section 2.3.7. Red cultivars were also analyzed for the same variables along with total anthocyanins, color, hue, and total phenols.

2.3.9 Analysis of wine

Samples of 100 mL of stored wine were collected from carboys prior to bottling. Analysis of pH and TA was performed for red and white cultivars (as described in section 2.3.7), followed of total anthocyanins, color, hue, and total phenols for red wines. Ethyl alcohol was analyzed in final wines by gas chromatography with a flame ionization detector (GC-FID; Agilent 6890, Wilmintong, Denmark) according the method of Nurgel et al. (2004). An internal standard solution of 0.5 mL of 99.4% 1-butanol (Fisher Scientific) in 500 mL of purified water was prepared. Each sample of wine was analyzed by duplicated mixing 50 μL of wine with 0.95 mL of 1-butanol solution and injected into the GC-FID with the following specifications: A capillary column (Agilent 122-7032 DB-Wax, 30 m x 250 μm diameter, 0.25 μm film thickness), carrier gas He at 179 mL/min split flow, and oven conditions: 60 ºC initial temp to 280 ºC final

temp, 4.46 min run time. The ethanol concentration was calculated using a regression formula obtained from calibration of known ethyl alcohol calibration using the reference factor (area of ethanol peak/area of internal standard; see Appendix Figure 2.1)

2.3.10 Statistical analysis

Results obtained were analyzed with SAS statistics software for analysis of variance to determine whether effects could exist between crop level treatments and hang time harvest during two years of experiment. Duncan´s test was used as a method of multiple comparisons to compare levels of group means (Lea et al. 1997).

2.4 Results

2.4.1 Pinot gris

Yield and berry composition (Table 2.4.1). In both years, the only variables that differed between crop levels were yield, clusters per vine, and crop load. In 2012, Brix also increased in half crop, but no other variables were affected.

Hang time led to more differences, with a 1.0 kg yield reduction between T0 and T2 in 2011 and 1.7 kg between the same times in 2012. Cluster weight was also reduced at T1 and T2 in 2011 with a final loss of 45 g per cluster from T0 to T2. In 2012 cluster weight decreased between T0 vs. T1 and T2 with the same final loss of 45 g. Hang time did not impact berry weight in 2011 and only between T0 and T1 in 2012. crop load decreased with increased hang time in both years; 2012 was 7 units higher at T0 than T2 in comparison with 2011 at the same hang time. There was an increase in Brix with respect to time, with a final 2º higher between T0 and T2 in 2011 and 5.4º higher between T0 and T2 in 2012 where was a slight increase in TA for both years. The pH was only affected in 2012 in T0 vs. T1 and T2, but there was no difference between T1 and T2.

Must composition (Table 2.4.2). No differences were detected between crop level treatments in 2011, but Brix increased 1º with crop level reduction in 2012.

For hang time Brix values, the trend in each year was similar. Brix differed between the first harvest (T0) and the other two harvests in 2011, whereby T1 and T2 were higher and ending with 3º higher at T2 with respect of T0. In 2012 there were increases at each of T1 and T2, with an increase of 7.5º in T2 with respect of T0. TA was impacted for both years with increases between times. However, in 2011 it just increased between T0 and T1 2.09 mg/L, followed by a reduction of 1.9 mg/L at T2. In 2012 the increase was constant with a final 1.86 mg/L higher at T2; a linear increase with hang time here was apparent. The pH was unaffected by hang time in 2011 but was higher in T2 with respect to T0 and T1 in 2012. In this last year mean pH values were higher than 2011.

Wine composition (Table 2.4.3). For 2011, no differences were found between crop levels for pH and TA, but ethanol concentration was higher by 0.5% in half crop wines. No crop level differences were observed in 2012.

All variables differed in 2011 and 2012 with respect to hang time. The TA increased in T1 and T2 compared to T0 in 2011 with a final 1.12 units at T2. The behaviour was different in 2012. Here the TA was lower at T0 in 2011 than 2012, almost half unit, but the mean value at T2 was similar (≈8 g/L) in both years. The pH increased between T0 and T1 vs. T2 in 2011 and a constant increase between times in 2012. Ethanol in 2011 also increased in T1 and T2 compared to T0; but the highest value here was at T2, greater than 1.6 %. In 2012 the increase was almost linear at each time with a final increase of 4.3% at T2 with respect to T0.

2.4.2 Riesling

Yield and berry composition (Table 2.4.4). For both years, reductions were found in yield, clusters per vine, and crop load between crop levels. Reducing crop also increased Brix in both years. TA was reduced only in 2012 with reduction of crop.

During 2011 all variables except vine size were different among hang times. Reductions in yield, cluster weight, berry weight, and crop load were observed with increased hang time. This trend was also observed during 2012, with most reductions occurring between T1 and T2 but not in T0. Berry weight

increased slightly between T0 and T1, followed by a reduction at T2 in both years. Brix had an increase of ≈ 2^{o} between T0 and T2 in both years. In 2011 a slight decrease in Brix was apparent between T0 and T1. With respect to TA**,** in 2011 T0 was lower than T2 but not T1, which was higher than T2. In 2012, TA behaviour had a decrease at T1 followed by an increase in T2. A higher TA was observed in 2011 with respect to 2012. The pH in both years displayed a slight increase from T0 to T1 followed by a decrease at T2. Interactions between hang time and crop levels were observed in yield at both years, while clusters per vine in 2011 and berry weight and berry pH in 2012.

Must composition (Table 2.4.5). For 2011 there were no differences between crop level treatments. In 2012 decreasing crop level increased must pH and Brix.

With respect to hang time all variables displayed differences in both years. In 2011, pH decreased between T0 and T1 but then increased at T2. The same behaviour was detected for Brix. In 2012 pH showed incremental increases with increased hang time, as did Brix. TA in 2011 had a reduction in T1 and T2 relative to T0. In 2012, TA decreased incrementally with increased hang time. Between years, 2012 had higher pH values and lower TA, Brix at T2 was 3.3^{o} higher in 2012 than 2011 where the most substantial increase was between T1 and T2. Interactions between hang time and crop levels were observed for must pH in 2012.

Wine composition (Table 2.4.6). For crop level, just ethanol was different in both years, with an increase of 0.6 % with reduction of crop in both years. For hang time, pH increased incrementally with time in 2011 and 2012. TA was not affected by hang time in 2011 but showed small decreases in 2012. Ethanol followed the same trend as Brix in the must, with an increase in both years at T2 with respect to T0 and T1. Interactions between hang time and crop levels were observed for wine pH in 2012.

2.4.3 Cabernet franc

Yield and berry composition (Table 2.4.7). In both years, variables that were different between crop treatments included yield, clusters per vine, and crop

load; all decreased with crop level reductions. Additionally, cluster weight, Brix (2011 only), and color (2011 only) increased with crop level reduction. During the second year, berry weight and total anthocyanins were slightly different with a decrease and increase, respectively, with crop reduction. A tendency towards increased anthocyanins with reduced crop level in 2011 was also observed.

Most variables differed between hang times in 2011 with the exception of clusters per vine and vine size. Decreased yield, cluster weight, berry weight, and crop load were observed with increased hang time. Both pH and Brix increased incrementally with increased hang time, but substantial reductions in anthocyanins and color, and concomitant increases in hue and phenols, were apparent in T2 berries. In 2012, there were once again incremental decreases in yield, clusters per vine, berry weight, and Ravaz Index with increased hang time. The pH decreased slightly with increased hang time while both TA and Brix increased. TA decreased in T2 preceded by an increase between T0 and T1 in 2011; that difference was not observed in 2012 where all concentrations at all hang times remained without substantial changes. Brix increased almost linearly with hang time in both years.

Must composition (Table 2.4.8). For 2011 only Brix, color and total phenols concentration were higher in half crop musts. In 2012, both Brix and pH increased and TA decreased with crop level reduction. All variables except for total phenols had differences between hang times in 2011; Brix and pH increased incrementally with hang time, as did both color and hue, but increases in anthocyanins and phenols at T1 were followed by sharp declines at T2. In 2012, increases in pH and Brix and decreases in TA were observed with increased hang time. In general, pH increased while TA was reduced in both years with increasing hang time. Brix increased with hang time in both years, with the exception of full crop T2 in 2011, anthocyanins in 2012 had a high increase at T2 with respect to T0 and T1, color and phenols had an increment linked to the reduction in hue during the time.

Wine composition (Table 2.4.9). For 2011, reduced crop level led to an increase in color intensity. In 2012 there was one effect on hue over wine

composition. With respect of hang time, all the variables changed at each time in both seasons; in 2011 the pH and ethanol increased incrementally with hang time and TA decreased. Anthocyanins and color decreased with hang time, with concomitant increases in hue, while phenols increased between T0 and T1 and then decreased in T2 wines. In 2012, pH and ethanol increased and TA decreased incrementally with hang time. The range in pH in 2012 was much less than in 2011. The TA dropped in 2011 at T2, which was not consistent with 2012 patterns. Anthocyanins and color reduced, the last had an increase between T0 and T1 reducing again in T2, with a concomitant increase in hue. In general, total phenols increased in 2012 from T0 to T1, with just a slight reduction observed between T1 and T2.

2.4.4 Cabernet Sauvignon

Yield and berry composition (Table 2.4.10). 2011 represented a year with few differences in almost all variables for crop level treatments except for decreases in yield, clusters per vine, cluster weight, and crop load, and increases in anthocyanins with reduced crop level. Same trends observed in 2012. Incremental decreases were observed in 2011 in yield, clusters per vine, cluster weight, berry weight, and crop load increased hang time, while pH and Brix increased. Very minor changes in TA occurred across hang times. As with Cabernet franc, noteworthy decreases were observed for anthocyanins, color, and phenols between T1 and T2. In 2012, bird predation prevented collection of yield data for the T2 treatment; nonetheless decreases were observed in the T1 treatment in terms of yield, clusters per vine, crop load and berry weight with increased hang time. Increases in Brix and TA and a small increase in pH were also observed in T1. TA and pH again had substantial differences between the years at T2; in the first year, an increase of pH at T2 was correlated with a drop in TA, a trend that was opposite in 2012, where the pH dropped in T2 after an increase between T0 and T1, which was reflected in a rise in TA.

Must composition (Table 2.4.11). No crop level effects on must composition were observed in 2011. In 2012, reduced crop level increased pH and Brix and reduced TA; a reduction was also observed in anthocyanins with

crop reduction. The pH in both years increased incrementally between hang times, which correlated with decreases in TA. Brix followed the same increasing trend with hang time in both years with higher levels in 2012. All variables except for color were different between hang times in 2011; as with berries there were decreases in anthocyanins and phenols at T2 with concomitant increases in hue.

Wine composition (Table 2.4.12). No differences were found between crop levels in 2011, and only a small pH increase for half crop was noticed in 2012. Color and hue had small differences, with color decreasing with a reduction in crop and a concomitant increase in hue. All variables were different between hang times in 2011 and all but ethanol and pH differed in 2012. The pH in both years followed incremental increases with hang time, as did ethanol (2011) and TA (2012). TA in 2011 decreased in the T2 treatment. The pH increased both years with the exception of full crop 2012; however, TA followed different trends each year with an increase in 2012 and a decrease in 2011. The ethanol concentration was higher from the beginning of 2012 in comparison with 2011. In both years, anthocyanins, color and phenols decrease in concentration with a concomitant increase in hue. In both years and increase in phenols between T0 and T1 was observed in phenols, with a reduction by T2.

2.5 Discussion

Weather conditions

The weather conditions in the vintages 2011 and 2012 were remarkably different and could be responsible in differences in grape and wine composition. In the 2011 harvest period, continuous rain was observed between 20 September and 1 November (Figure 2.3). Harvest of Pinot gris for all hang times was the most impacted by rain followed by Riesling. This could explain why no differences were detected in variables like berry weight in this cultivar (Table 2.4.1). During this same period of time, mean temperatures reduced from 15º to 10ºC. For the period from November 2, to December 6, drier conditions were experienced, but with rain during the last week of November; this linked with the temperature reduction from 15º to 10ºC could explain the possible spoilage of

grapes of red cultivars at the last hang time. Therefore, 2011 was considered a wet/warm vintage during the whole harvest period.

In 2012 during the harvest period, less rain occurred between hang times of cultivars like Pinot gris (Figure 2.5). Riesling on the other hand, and particularly at the last stage, had an increase of rain during the last week of October. Completely dry conditions were followed in November, affecting particularly the last hang times for Cabernet franc and Cabernet Sauvignon. It is important to point out that during this experimental phase in this year, the decrease in temperature was substantially different than the previous year, starting with days > 20ºC at the beginning of harvest to temperatures ≈ 0ºC in December (Figure 2.6). Therefore 2012 was considered a more dry/cold year in comparison with 2011. The harvest at each year was also different with a tendency for an earlier beginning in 2012 than in 2011 for each cultivar (Table 2.5); a dry/hot year during the summer until harvest such as in 2012 explains why all cultivars easily reached maturity for commercial harvest.

Effects of crop reduction

As expected, reduction in yield, clusters per vine and crop load, and an increase in Brix occurred in all cultivars in both years in cluster-thinned treatments, which is consistent with prior studies (Berkey et al. 2011; Freeman and Kliewer 1983; Reynolds et al. 1994).

Reynolds et al. (1994) reported that early season cluster thinning led to slightly less yield per vine, but higher cluster weights, berries per cluster and berry weights. In the case of this experiment, cluster thinning occurred close to veraison, and consequently an increase in cluster weight was only observed in Cabernet franc in both years, while berry weight was slightly decreased or remained unchanged.

Even though Pinot gris had an increase in berry weight, this was not substantial enough to increase cluster weight. This increase could be explained by the capacity that the vine has to self-regulate after fruit thinning (Freeman and Kliewer 1983) or by simply by the tendency of the basal cluster to be larger than apical clusters removed during thinning (Di Profio et al. 2011a).

Vine size was always inexplicably reduced slightly in cluster-thinned vines, and only in Cabernet franc in 2012 was a slight increase observed consistent with Bravdo et al. (1984) and others. What is important to consider was the crop load. In this experiment, all crop loads were reduced with cluster thinning, and in all cases, FC and HC, they were < 12. It has been reported that crop loads > 12 produce conspicuous effects of overcropping with a reduction of wine quality, color, intensity, delay of maturation and sugar accumulation (Bravdo et al. 1984; Edson et al. 1995).

Pinot gris in 2012 was the only cultivar that had a value > 12 in the full crop treatment, suggesting that overcropping occurred, and this was linked to the high yield observed in that treatment, which was twice that of the previous year with a lower vine size.

The berry TA was always reduced with crop reduction particularly in Riesling in 2012. This phenomenon suggests accelerated fruit maturity. The reduction in TA may be explained primarily as a reduction in malic acid in the berries associated with temperature. The basal clusters were typically more exposed to sunlight than apical clusters as a result of basal leaf removal, and hence a reduction in malic acid was somewhat expected (Boulton 1980).

Results between must and wine reflected the effects obtained in the berries. It has been reported that the effect of crop levels is consistent for most constituents and could be transferred into the wines (Weaver et al. 1961). Some results could be correlated between those obtained from samples of grape and must.

In general, Brix, TA, and pH followed the same pattern between berries, must and wine with an increase, decrease and increase, respectively, when crop reduction was applied. Cluster thinning effects on berry pH and TA were rarely substantive, with the exception of Riesling in 2012, a year that was considered cold and dry during harvest (Figure 2.3). The mean TA values here were 1 g/L below those obtained in 2011. These results are consistent with others (Freeman and Kliewer 1983), who observed no impact on TA and pH in thinned vines. Ough and Nagaoka (1984) also reported an increase in pH, TA and Brix at the

same harvest date but no differences in TA or pH. The increase in concentration of ethanol in wine was linked to the increase in berry Brix with reduced crops. Crop reduction typically increases the concentration of ethanol in final wines (Di Profio et al. 2011b; Jackson and Lombard 1993; Reynolds et al. 1996), consistent with this experiment where all the cultivars produced an increase in ethanol in reduced crop treatments. For the particular case of Cabernet Sauvignon in both years, a slight reduction was observed but not sufficient to be considered.

Increases in total phenols and anthocyanins as well as color intensity in grape juice normally occur when reductions in crop level are imposed in red cultivars (Di Profio et al. 2011a,b; Mazza et al. 1999). Berries, must and wine of cluster-thinned Cabernet franc in this experiment had in general an increase in total anthocyanins, color and total phenols consistent with the results finding in literature.

Particular differences could be observed between vintages in both cultivars; although Brix levels were very close between years, they were ≈ 1^{o} Brix higher in 2012, while pH values were lower in 2012 than 2011. This lower pH is linked to higher tannins, extracts and color (Weaver et al. 1961), which is correlated with the increase in concentration of total phenols and anthocyanins in that year compared with the previous. Cabernet Sauvignon berries had the same pattern in both years, as did Cabernet franc. The must, however, had a decrease in anthocyanins with cluster thinning in both years, possibly due to effect of dilution or lack of extraction after pressing that was reflected directly in the wines.

Effects of hang time.

In all cultivars, reductions in yield were linked to reductions in cluster weight and berry weight obtained at each extended hang time (T1 and T2) in both years. This reduction was expected due to desiccation associated with the over-ripening process (Chkaiban et al. 2007; Constantini et al. 2006; Moreno et al. 2008). It is possible that reduction in cluster weight was also due to actual loss of berries (i.e. abscission) rather than desiccation.

The dehydration of grapes was concomitant with the increase of Brix in all cultivars at each extended hang time; this increase was also expected in accordance with the literature (Constantini et al. 2006). This increase was reflected in must and directly proportional with an increase of ethanol content, which also increased. Differences in ethanol concentration could be associated with differences in berry Brix (Moreno et al 2008).

Vine size as expected was never affected by extended hang time but a pattern was apparent in all cultivars between vintages involving a reduction in vine size from 2011 to 2012, particularly if one observes the half and full crop X hang time combinations (Tables 2.4.13, 2.4.15, 2.4.17 and 2.4.20). Therefore, a possible effect linked to crop reduction plus extended hang time may have affected the physiology of the vine, eliciting a vine size reduction between years, but not between individual treatments. The reduction in Ravaz Index was directly proportional to the reduction of yield rather than vine size. It is important to note that because yield reductions in extended hang time treatments were associated with desiccation, bird predation, and other factors, any increase in Ravaz Index in these treatments would not have true physiological significance.

It has been reported that the process of dehydration of grapes is also linked to an increase of TA (Bellincontron et al. 2004). This trend was observed in all cultivars in almost all the treatments with some exception like Cabernet franc in 2011, where an increase in TA was observed just between the two first hang times, followed by a substantial reduction. This decline in berry TA has been reported as a possible change in the acid composition in berries (Freeman and Kliewer 1983). Malic acid is consumed during the first step of dehydration but as dehydration continues this could mask the malate loss, plus sugars can also be synthetized from malic acid by gluconeogenesis at the last stage of the slow grape dehydration (Amati et al. 1983; Corte et al. 2001). This could explain the phenomenon involving a reduction of TA followed by an increase, which was also observed during both years in Cabernet franc. Changes in TA were evident in musts from Riesling, Cabernet franc and Cabernet Sauvignon. During both years, a reduction was observed at each extended hang time with a concomitant

increase of pH, a trend that was reflected in the final wines. Cabernet franc and Cabernet Sauvignon wines also displayed reductions in TA in both years with extended hang time, but these trends were observed only in 2011. In 2012, on the other hand, there was an increase in TA with extended hang time, particularly T2. This increase could be related with vinegary spoilage from acetic acid bacteria, e.g. *Gluconobacter oxydants, Acetobacter pasteurianus* and *A. aceti* (Sponholz 1993). Not only will increases in acetic acid, i.e. volatile acidity, raise TA substantially, but also increases in propionic, hexanoic, and formic acids (Sponholz 1993) can generate a change in the final wine TA (increase due to volatile acidity). Acetic acid bacteria are also reported as responsible for oxidation of ethanol to acetic acid and acetaldehyde (Fleet 1993). This oxidation of ethanol is apparent with the reduction of ethanol concentration at the last hang time in Cabernet Sauvignon.

Increases in concentrations of total phenols and anthocyanins were observed in tunnel-treated Sangiovese grapes (Moreno et al. 2008). Similar increases were observed in this trial in Cabernet franc berries for total phenols in both years, but for Cabernet Sauvignon this increase was only present between the first two hang times (T0 and T1) with a decrease in T2. Contrary to the literature was the reduction in total anthocyanins with extended hang time. A possible polymerization with other phenols could have occurred (Bakker and Clarke 2012; Singleton and Rossi 1998). When a loss of water of around 0.5% occurs in grape, the cell wall enzyme activity is increased. At the same time, a change or reduction of polyphenol levels occurs not only due to concentration but even to change of metabolisms (Bellincontron et al. 2004; Constantini et al. 2006; Zamboni et al. 2008). This also could explain the reduction of anthocyanins in these red cultivars. Color in berries also decreased, which is linked to the reduction in anthocyanins; i.e. they contribute highly to the red color in wines (Bakker and Clarke 2012).

Must and wine in Cabernet franc and Cabernet Sauvignon had similar patterns between them, but here the concentration of anthocyanins behaved in a different way. In 2011 reduction for anthocyanins at T2 was linked to an increase

of pH at the same time, generating polymerization or precipitation. Warm temperatures (between 5 º to 15 ºC) following T1 also affected anthocyanin concentrations (Singleton and Rossi 1965) (Figure 2.2), whereas with 2012 there were more days < 5ºC (Figure 2.4) following T1. The 2012 season was more stable in terms of pH, with just an increase up pH 3.38 for Cabernet franc and pH 3.35 for Cabernet Sauvignon. Following extended hang time, a more stable conformation of anthocyanins occurred, since at pH < 3.5, the flavylium ion (red) form with a maximum near 520 nm will be enhanced (Singleton 1998). Wines of Cabernet franc were higher in pH particularly at T2, accompanied by a reduction in anthocyanins and color and increase of hue; higher pH in wine typically elicits a dull color (Hepner and Bravdo 1985). The same trend was observed in wines of Cabernet Sauvignon, and differences also occurred relative to season, whereby 2011 was lower in anthocyanins with an accompanying higher pH, in comparison with 2012 where pH values were < 3.5 with higher concentrations of anthocyanins and color.

Comparing Hang time vs. Crop reduction.

In all cultivars some interactions were significant between crop reduction and hang time, but were more apparent between vintages. For the case of Pinot gris and Riesling, no differences were detected between mean values of full crop at each hang time and half crop at each time (Tables 2.4.13 to 2.4.16). Yield in FC/T2 was closer to the value obtained for HC/T0, suggesting that the reduction in yield from crop reduction will be equal to 6 weeks of extended hang time without crop reduction. Similar behaviour was observed for Brix, whereby a delay in maturation occurred, particularly in 2012 for the full crop treatment, and it was not until 3 weeks after regular harvest that the values of Brix were similar to the regular harvest for half crop treatments. This particular delay in maturation could be related to an apparent lack of lignification of the rachises in commercial harvest (T0), eliciting a continued vascular connection of the berry with the vine, therefore a real maturation is reached after 3 weeks (T1).

No crop level X hang time interactions were detected in berry, must and wine variables, which suggests that use of extended hang time does not impact

substantially on characteristics other than yield and berry/must Brix, (and subsequent wine ethanol production) when cluster thinning alone was compared to extended hang time plus crop reduction. Differences here were more marked between vintages where 2011, a wetter year during the harvest period with higher temperatures, resulted in lower Brix and high TA compared with 2012, a drier year that was cooler during the harvest period.

For Cabernet franc, the same trend was observed for yield in 2011, but not in 2012 due to loss of fruit from predation. Berry and must Brix did not differ between crop levels. Anthocyanins did not improve with increase of hang time without crop reduction, nor did color and hue. Difference between vintages was more apparent here. A better color and higher concentration in anthocyanins linked with a lower pH in 2012 had the better impact in quality rather than delaying of fruit with the full crop. In must, a benefit in delaying harvest with full crop could be observed in 2011 at T1. Here the values of Brix were the same as harvest of FC/T0, with a closer value of anthocyanins but a higher value of hue, which suggests a more dull color. Must composition in 2012 did not differ between FC and HC with respect of time, suggesting that full crop combined with extended hang time did not compromise the quality of must and wine.

Cabernet Sauvignon (Table 2.4.16) had a substantial increase in yield in full crop for each hang time. Brix increased with extended hang time in T1 and T2, but no other important effects were detected in berries with the exception of decreased anthocyanins, color and phenols. In other words, leaving a full crop on the vine for an extended period had the same effects as cluster thinning without extended hang time, with the exception of a yield increase for the full crop treatment. The same trend occurred in 2012 but with an increase in Brix at T2 in full crop, with higher anthocyanins and color and a lower pH.

Extended hang time was beneficially equal in both crop levels for T0 and T1 in 2011. Increases in pH > 3.5 after T1 compromised the quality of both must and wine, while in 2012 there were better results in both full and half crops; there was higher Brix and ethanol, lower pH, higher TA, and higher anthocyanins and phenols in T1. This suggests that increasing crop does not compromise the

quality of must and wine if one extends harvest date up to 3 weeks after regular harvest.

Differences in vintage

Noticeable effects in some variables between vintages were present in grapes and wine. In general, higher values in Brix and lower pH in grapes during 2012 (dry and cold) regarding 2011 (warmer and wet/rainy) were found. The particular effect on pH allowed a higher extraction of phenols and anthocyanins that were reflected in both red cultivars in 2012. High concentration in Brix followed by high levels of alcohol in wine, particularly in red cultivars, also were linked to the weather conditions for 2012 (dry and cold). Rainy conditions during 2011 linked to sudden increase of temperature in particular days, could generate the presence and growing of spoilage microorganism particularly at the last stages of hang in all cultivars. A particular early commercial harvest was also observed during 2012 after a hot summer conditions in the mentioned year.

2.6 Conclusions

Viticultural treatments imposed by this study had impact over the composition of grapes and subsequent wine. It was hypothesized that keeping a full crop with extended hang time might have a greater impact on wine quality than to reduce the crop level. This increase in hang time led to a higher increase in Brix than crop reduction at a commercial harvest date. The major impact was that the yield was reduced at the same levels as crop reduction after 6 weeks of extended hang time in white cultivars and 3 weeks in red cultivars. Overcropping effects, if present, had few impacts on berry, must and wine composition, and those few cases of delayed maturity were overcome by delays of harvest. Effects of extended hang time were extrapolated to must and wine Increases in pH and TA had minimal effects on the overall quality other than reductions in polyphenols and color in red cultivars.

The effect on vintage was important for this experiment, since increases in Brix and yield were greater in 2012 with a better overall quality in wines, linked to the difference in climatic condition from fruit set until harvest. The 2011 season

was a wetter year with higher temperatures during harvest in comparison with a more dry and cool year during harvest in 2012.

Pinot gris and Riesling benefitted more from the extension of hang time than did the red cultivars. In fact, Cabernet franc wine was compromised in wine quality particularly at the last stage with major increases in pH and reductions in TA, anthocyanins, color and phenols. Cabernet Sauvignon also had a reduction in TA, anthocyanins and color but this cultivar benefitted more in 2012 with increments in constituents and in particular a lower pH, which is consistent with a better wine capable of preservation and aging. An important consideration particularly to the red cultivars is to prevent loss of fruit by predators particularly at the very late hang times.

This study provides with new knowledge about the effects on grape and wine with extension of harvest. Previous studies has been used post-harvest techniques, e.g. thermo tunnel dry out, to investigate this effect, but the difference on this experiment is that fruit is still in the vine with presence of canopy; therefore, the changes in morphology in vine could make a different impact over the constituent in grape that will be transported to the final wine.

With all the information obtained during this experiment, it can be concluded that in general, extension in harvest had a better impact in general quality in grape and wine than crop reduction. This is linked not only with higher concentration of sugars, but also to increase in yield that could be beneficial in the production of final wine. Is important to consider characteristics like climatic conditions and type of cultivar when extension of harvest is chosen as a viticultural treatment. Faster decisions in vineyard plus selection of the most resistant will also be necessary to ensure the best quality in wine.

2.7 Literature Cited

Amati A, Ferrarini R, Riponi C and Zironi R, (1983). Una nuova tecnologia per l'appassimento delle uve. *Vigne Vini* 10:27–35.

Bakker, J., and Clarke, R. J. (2012). Wine flavour chemistry (2nd ed.). Chichester, West Sussex; Ames, Iowa: Wiley-Blackwell

Balcar, J., and Hernandez, J. (1988). Translocacion de fotosintatos en sarmientos de la vid durante el periodo vegetativo. Vitis. 27:13-20.

Bell, A., Ough, C., and Kliewer, W. M. (1979). Effects on must and wine composition, rates of fermentation, and wine quality of nitrogen fertilization of *Vitis vinifera* var. Thompson Seedless grapevines. Am. J. Enol. Vitic. 30:124-129.

Bellincontro, A., De Santis, D., Botondi, R., Villa, I., and Mencarelli, F. (2004). Different postharvest dehydration rates affect quality characteristics and volatile compounds of malvasia, trebbiano and sangiovese grapes for wine production. J. Sci. Food Agr. 84:1791-1800.

Berkey, T. G., Mansfield, A. K., Lerch, S. D., Meyers, J. M., and Heuvel, J. E. V. (2011). Crop load adjustment in 'Seyval Blanc' Winegrape: Impacts on yield components, fruit composition, consumer wine preferences, and economics of production. Horttechnology, 21:593-598.

Boulton, R. (1980). The relationship between total acidity, titratable acidity and pH in grape tissue. Vitis 19:113-120.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., and Tabacman, H. (1984). Effect of crop level on growth, yield and wine quality of a high yielding Carignane vineyard. Am. J. Enol. Vitic. 35:247-252.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., and Tabacman, H. (1985). Effect of crop level and crop load on growth, yield, must and wine composition, and quality of Cabernet Sauvignon. Am. J. Enol. Vitic. 36:125-131.

Chkaiban, L., Botondi, R., Bellincontro, A., Santis, D. d., Kefalas, P., and Mencarelli, F. (2007). Influence of postharvest water stress on lipoxygenase and alcohol dehydrogenase activities, and on the composition of some volatile compounds of Gewürztraminer grapes dehydrated under controlled and uncontrolled thermohygrometric conditions. Aus. J. G. W. Res. 13:142-149.

Constantini, V., Bellincontro, A., de Santis, D., Botondi, R., and Mencarelli, F. (2006). Metabolic changes of Malvasia grapes for wine production during postharvest drying. J. Agr. Food Chem. 54:3334-3340.

Corte V, Oliva D, Ragusa M, Genna G, Strano M and Di Stefano R, (2001). Aspetti tecnici, microbiologici e chimici connessi con i sistemi di appassimento delle uve. *Enologo* 12:87–97.

Di Profio, F., Reynolds, A. G., and Kasimos, A. (2011a). Canopy management and enzyme impacts on Merlot, Cabernet franc, and Cabernet sauvignon. I. yield and berry composition. Am. J. Enol. Vitic. 62:139-151.

Di Profio, F., Reynolds, A. G., and Kasimos, A. (2011b). Canopy management and enzyme impacts on Merlot, Cabernet franc, and Cabernet Sauvignon. II. Wine composition and quality. Am. J. Enol. Vitic. *62*:152-168.

Edson, C. G., Howell, G., and Flore, J. (1993). Influence of crop load on photosynthesis and dry matter partitioning of Seyval grapevines I. single leaf and whole vine response pre-and post-harvest. Am. J. Enol. Vitic. 44:139-147.

Edson, C. G., Howell, G. S., and Flore, J. (1995). Influence of crop load on photosynthesis and dry matter partitioning of seyval grapevines. III. seasonal changes in dry matter partitioning, vine morphology, yield, and fruit composition. Am. J. Enol. Vitic*. 46*:478-485.

Fang, F., Li, J., Zhang, P., Tang, K., Wang, W., Pan, Q., and Huang, W. (2008). Effects of grape variety, harvest date, fermentation vessel and wine ageing on flavonoid concentration in red wines. Food Res. Int. 41:53-60.

Fischer, U., Roth, D., and Christmann, M. (1999). The impact of geographic origin, vintage and wine estate on sensory properties of "*Vitis vinifera*" cv. Riesling wines. Food Qua. Pref. 10:281-288.

Fisher, K. H., Bradt, O., Wiebe, J., and Dirks, V. (1977). Cluster-thinning "De chaunac" French hybrid grapes improves vine vigor and fruit quality in Ontario. J. Am. Soc. Hort. Sci. 102:162-165.

Fleet, G. H. (1993). Wine: Microbiology and Biotechnology CRC Press. Sydney Australia

Freeman, B. M. and Kliewer, M. (1983). Effect of irrigation, crop level and potassium fertilization on carignane vines. II. grape and wine quality. Am. J. Enol. Vitic*.* 34:197-207.

Fuleki, T. And Francis, F. J. (1968). Quantitative methods for anthocyanins. J. Food Sci. 33:266-274.

Hale, C. (1977). Relation between potassium and the malate and tartrate contents of grape berries. Vitis 16:9-19.

Haynes, S. J. (2000). Geology and wine 2. A geological foundation for terroirs and potential sub-appellations of niagara peninsula wines, ontario, canada. Geoscience Canada. 27:67-87.

Hepner, Y. and Bravdo B. (1985). Effect of crop level and drip irrigation scheduling on the potassium status of Cabernet Sauvignon and Carignane vines and its influence on must and wine composition and quality. Am. J. Enol. Vitic. 36:140-147.

Herbert, P., Cabrita, M. J., Ratola, N., Laureano, O., and Alves, A. (2005). Free amino acids and biogenic amines in wines and musts from the Alentejo region. evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage. J. Food Eng. 66:315-322.

Himelrick, D. G. (2003). Handling, storage and postharvest physiology of Muscadine grapes: A review. Small Fruits Review 2:45-62.

Jackson, D., and Lombard, P. (1993). Environmental and management practices affecting grape composition and wine quality-a review. Am. J. Enol. Vitic. 44:409-430.

Jones, G. V., and Davis, R. E. (2000). Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. Am. J. Enol. Vitic. 51:249-261.

Kataoka, I., Kubo, Y., Sugiura, A., and Tomana, T. (1984). Effects of temperature, cluster shading and some growth regulators on L-phenylalanine ammonia-lyase activity and anthocyanin accumulation in black grapes. Memoirs of the College of Agriculture-Kyoto University, 124:35-72.

Keller, M., Mills, L. J., Wample, R. L., and Spayd, S. E. (2005). Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. Am. J. Enol. Vitic. 56:91-103.

Kingston, M. S., Presant, E. W. (1989) The soils of the regional municipality of Niagara. Ontario Institute of Pedology, Rep. 60 Vol. 1 and 2.

Kliewer, W. M. (1971). Effect of day temperature and light intensity on concentration of malic and tartaric acids in Vitis vinifera L. grapes. J. Am. Soc. Hort. Sci. 96:372-377.

Kliewer, W. M. (1977). Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. Am. J. Enol. Vitic. 28:96-103.

Kliewer, W. M., and Dokoozlian, N.K. (2005). Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. Am. J. Enol. Vitic. 56:170-181.

Kliewer, W. M., Howarth, L., and Omori, M. (1967). Concentrations of tartaric acid and malic acids and their salts in *Vitis vinifera* grapes. Am. J. Enol. Vitic. 18:42-54.

Kliewer, W. M., and Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. Am. J. Enol. Vitic. 23:71-77.

Kliewer, W., and Weaver, R. (1971). Effect of crop level and leaf area on growth, composition, and coloration of Tokay grapes. Am. J. Enol. Vitic. 22:172-177.

Lakso, A. N., and Kliewer, W. M. (1978). The influence of temperature on malic acid metabolism in grape berries. II. temperature responses of net dark CO_2 fixation and malic acid pools. Am. J. Enol. Vitic. 29:145-149.

Lea, P., Naes, T., and Rødbotten, M. (1997). Analysis of variance for sensory data. Wiley and Sons. West Sussex, England.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., and Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. J. Agr. Food Chem. 10:4009-4017.

McCarthy, M. (1999). Weight loss from ripening berries of Shiraz grapevines (*Vitis vinifera* L. cv. Shiraz). Austral. J. Grape Wine Res. 5:10-16.

Molitor, D., Behr, M., Hoffmann, L., and Evers, D. (2012). Impact of grape cluster division on cluster morphology and bunch rot epidemic. Am. J. Enol. Vitic. 63:508-514.

Moreno, J. J., Cerpa-Calderón, F., Cohen, S. D., Fang, Y., Qian, M., and Kennedy, J. A. (2008). Effect of postharvest dehydration on the composition of Pinot noir grapes *Vitis vinifera* L. and wine. Food Chem. 109:755-762.

Morris, J., and Cawthon, D. (1982). Effect of irrigation, fruit load, and potassium fertilization on yield, quality, and petiole analysis of Concord (*Vitis labrusca* L.) grapes. Am. J. Enol. Vitic. 33:145-148.

Mortensen, J. (1986). Effect of bunch and grape maturity on finished Muscadine wine. Proc. Fla. State Hort. Soc. 99:194-200.

Nurgel, C., Pickering, G. J., and Inglis, D. L. (2004). Sensory and chemical characteristics of canadian ice wines. J. Sci. F. Agri. 84:1675-1684.

Ough, C., and Nagaoka, R. (1984). Effect of cluster thinning and vineyard yields on grape and wine composition and wine quality of Cabernet Sauvignon. Am. J. Enol. Vitic. 35:30-34.

Pigott, S. (1992). Riesling. Viking Pr. London

Ravaz, L., and Sicard, L. (1903). Sur la brunissure de la vigne. CR Acad. Sci. 136:1276-1278.

Reynolds, A. G., Pool, R. M., and Mattick, L. R. (1986a). Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. Vitis 25:85-95.

Reynolds, A. G., Pool, R. M., and Mattick, L. R. (1986b). Effect of shoot density and crop control on growth, yield, fruit composition, and wine quality of 'Seyval blanc' grapes. J. Am. Soc. Hortic. Sci. 111:55-63.

Reynolds, A. G., Price, S. F., Wardle, D. A, and Watson, B. T. (1994). Fruit environment and crop level effects on Pinot noir. I. vine performance and fruit composition in British Columbia. Am. J. Enol. Vitic. 45:452-459.

Reynolds, A. G., and Wardle, D. A. (1989). Impact of various canopy manipulation techniques on growth, yield, fruit composition, and wine quality of Gewürztraminer. Am. J. Enol. Vitic. 40:121-129.

Reynolds, A. G., Yerle, S., Watson, B. T., Price, S. F., and Wardle, D. A. (1996). Fruit environment and crop level effects on Pinot noir. III. composition and descriptive analysis of Oregon and British Columbia wines. Am. J. Enol. Vitic. 47:329-339.

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16:144-158.

Sponholz, W. (1993). Wine spoilage by microorganisms. *In* Wine Microbiology and Biotechnology, Fleet (Ed), pp. 395-420. Harwood Academic Publisher, Chur, Switzerland.

Vine and Tree Fruit Innovations (2013). www.vineinnovations.com. Weather INovations, Ontario, Canada. Review September 2013

VQA, Vintner´s Quality Alliance Ontario, Four Mile Creek, (2013). www.vqaontario.com/Appellations/NiagaraPeninsula/FourMileCreek. VQA Ontario, Canada. Review September 2013.

Waterhouse, A. (2006). Folin-ciocalteau micro method for total phenol in wine. www.waterhouse.Ucdavis.edu/phenol/folinmicro.Htm,(Accessed: May 2011)

Weaver, R. J., and McCune, S. B. (1960). Effects of overcropping Alicante Bouschet grapevines in relation to carbohydrate nutrition and development of the vine. Proc. Am. Soc. Hortic. Sci. 75:341-353.

Weaver, R. J., McCune, S. B., and Amerine, M. A. (1961). Effect of level of crop on vine behavior and wine composition in Carignane and Grenache grapes. Am. J. Enol. Vitic. 12:175-184.

Weaver, R. J., and Pool, R. M. (1968). Effect of various levels of cropping on *Vitis vinifera* grapevines. Am. J. Enol. Vitic. 19:185-193.

Winkler, A. J. (1954). Effects of overcropping. Am. J. Enol. Vitic. 5:4-12.

Zamboni, A., Minoia, L., Ferrarini, A., Tornielli, G. B., Zago, E., Delledonne, M., and Pezzotti, M. (2008). Molecular analysis of post-harvest withering in grape by AFLP transcriptional profiling. J. Exp. Bot. 59:4145-4159.

Appendix Chapter 2

Table 2.4.1 Impact of hang time and crop level treatments on yield and berry composition of Pinot gris grapes, 2011-2012.

Year	Factor	Yield /vine	Clusters/ vine	Cluster weight (g)	Berry weight (g)	Vine size (kg)	Crop load	pH	Titratable acidity (g/L)	Brix
2011	**Crop Level**									
	Full	2.8	31	90.5	1.22	0.53	6.5	3.48	6.34	24.2
	Half	1.9	21	93.0	1.22	0.50	4.7	3.45	6.26	24.3
	Significance[a]	****	****	ns	ns	ns	***	ns	ns	ns
	Hang time									
	T0	2.9 a	25	118.0 a	1.24	0.51	7.1 a	3.48	6.32 a	23.2 c
	T1	2.3 b	27	84.0 b	1.22	0.50	5.4 b	3.47	6.07 b	24.4 b
	T2	1.9 c	27	72.8 c	1.21	0.54	4.3 b	3.44	6.51 a	25.2 a
	Significance[a]	****	ns	****	ns	ns	****	ns	**	****
	Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns
2012	**Crop Level**									
	Full	4.7	43	110.8	1.26	0.43	13.5	3.53	5.99	22.6
	Half	3.2	29	112.2	1.21	0.4	10.1	3.54	5.89	23.7
	Significance[a]	****	****	ns	ns	ns	***	ns	ns	****
	Hang time									
	T0	4.9 a	36	137.9 a	1.29 a	0.40	14.9 a	3.49 b	5.38 b	20.5 c
	T1	3.6 b	36	102.7 b	1.24 ab	0.42	10.9 b	3.55 a	6.40 a	23.1 b
	T2	3.2 c	36	92.9 b	1.20 b	0.42	9.4 b	3.56 a	6.04 a	25.9 a
	Significance[a]	****	ns	****	*	ns	****	***	***	****
	Interaction	*	ns	ns	ns	ns	ns	ns	ns	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test

Table 2.4.2 Impact of hang time and crop level treatments on the must composition of Pinot gris 2011-2012.

Year	Factor	pH	Titratable acidity (g/L)	Brix
2011	**Crop Level**			
	Full	3.12	8.09	24.0
	Half	3.15	7.99	24.4
	Significance[a]	ns	ns	ns
	Hang time			
	T0	3.18 a	7.28 b	22.3 b
	T1	3.13 ab	9.37 a	25.0 a
	T2	3.10 b	7.47 b	25.3 a
	Significance[a]	ns	***	***
	Interaction	ns	ns	ns
2012	**Crop Level**			
	Full	3.23	6.88	22.6
	Half	3.26	6.74	23.6
	Significance[a]	ns	ns	*
	Hang time			
	T0	3.22 b	6.02 c	19.6 c
	T1	3.21 b	6.52 b	22.6 b
	T2	3.31 a	7.88 a	27.1 a
	Significance[a]	****	****	****
	Interaction	ns	ns	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively.

Mean values with same letters are not significantly different at p≤ 0.05 by

Duncan´s multiple range test.

Table 2.4.3 Impact of hang time and crop level treatments on the wine composition of Pinot gris 2011-2012.

Year	Factor	pH	Titratable acidity (g/L)	Ethanol (% v/v)
2011	**Crop Level**			
	Full	3.13	7.87	13.7
	Half	3.15	7.74	14.2
	Significance[a]	ns	ns	*
	Hang time			
	T0	3.06 b	7.10 b	13.1 c
	T1	3.11 b	8.09 a	14.7 a
	T2	3.26 a	8.22 a	13.9 b
	Significance[a]	****	**	****
	Interaction	ns	ns	ns
2012	**Crop Level**			
	Full	3.08	7.30	14.1
	Half	3.10	6.91	14.4
	Significance[a]	ns	ns	ns
	Hang time			
	T0	2.92 c	6.67 b	12.3 c
	T1	3.04 b	6.65 b	14.0 b
	T2	3.32 a	8.00 a	16.6 a
	Significance[a]	****	****	****
	Interaction	ns	ns	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

Table 2.4.4 Impact of hang time and crop level treatments on the yield and berry composition of Riesling 2011-2012.

Year	Factor	Yield/ vine	Clusters/ vine	Cluster weight (g)	Berry weight (g)	Vine size (kg)	Crop load	pH	Titratable acidity (g/L)	Brix
2011	**Crop Level**									
	Full	2.9	37	77.9	1.36	0.44	8.0	3.24	8.40	19.9
	Half	1.7	23	74.6	1.33	0.41	4.9	3.23	8.39	20.3
	Significance[a]	****	****	ns	ns	ns	****	ns	ns	**
	Hang time									
	T0	3.1 a	34 a	91.5 a	1.40 a	0.45	7.3 a	3.23 ab	8.21 b	19.7 c
	T1	2.5 b	32 b	77.6 b	1.41 a	0.42	8.0 a	3.26 a	8.58 a	19.3 b
	T2	1.5 c	25 c	59.7 c	1.23 b	0.40	4.2 b	3.21 b	8.40 ab	21.3 a
	Significance[a]	****	****	****	****	ns	****	*	**	****
	Interaction	****	**	ns	ns	ns	ns	ns	ns	ns
2012	**Crop Level**									
	Full	4.0	38	104.5	1.49	0.37	11.7	3.31	7.70	19.7
	Half	2.2	22	98.6	1.44	0.36	7.6	3.33	7.33	21.1
	Significance[a]	****	****	ns	ns	ns	****	ns	****	****
	Hang time									
	T0	3.7 a	33 a	112.0 a	1.55 a	0.36	10.9 a	3.32 ab	7.38 b	19.4 c
	T1	3.6 a	32 a	115.1 a	1.57 a	0.35	11.9 a	3.35 a	7.13 c	20.0 b
	T2	2.0 b	26 b	77.4 b	1.28 b	0.38	6.2 b	3.30 b	8.07 a	21.6 a
	Significance[a]	****	****	****	****	ns	****	**	****	****
	Interaction	**	ns	ns	****	ns	ns	*	ns	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

Table 2.4.5 Impact of hang time and crop level treatments on the must composition of Riesling 2011-2012.

Year	Factor	pH	Titratable acidity (g/L)	Brix
2011	**Crop Level**			
	Full	2.90	8.89	21.8
	Half	2.90	8.52	22.1
	Significance[a]	ns	ns	ns
	Hang time			
	T0	2.87 b	10.00 a	20.2 b
	T1	2.79 c	8.01 b	20.0 b
	T2	3.01 a	8.10 b	25.6 a
	Significance[a]	****	****	****
	Interaction	ns	ns	ns
2012	**Crop Level**			
	Full	3.12	7.39	20.5
	Half	3.15	7.18	21.7
	Significance[a]	*	ns	****
	Hang time			
	T0	3.09 c	7.73 a	20.0 c
	T1	3.13 b	7.46 a	21.3 b
	T2	3.19 a	6.67 b	21.9 a
	Significance[a]	****	****	****
	Interaction	****	ns	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

Table 2.4.6 Impact of hang time and crop level treatments on the wine composition of Riesling 2011-2012.

Year	Factor	pH	Titratable acidity (g/L)	Ethanol (% v/v)
2011	**Crop Level**			
	Full	2.88	9.35	12.9
	Half	2.90	9.16	13.5
	Significance[a]	ns	ns	*
	Hang time			
	T0	2.73 c	9.64	12.1 b
	T1	2.87 b	9.05	12.2 b
	T2	3.08 a	9.08	15.3 a
	Significance[a]	****	ns	****
	Interaction	ns	ns	ns
2012	**Crop Level**			
	Full	2.89	7.58	12.8
	Half	2.90	7.27	13.4
	Significance[a]	ns	ns	**
	Hang time			
	T0	2.85 c	7.88 a	12.7 b
	T1	2.89 b	7.37 b	13.2 a
	T2	2.94 a	7.03 b	13.4 a
	Significance[a]	***	**	**
	Interaction	*	ns	ns

[a]*,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

Table 2.4.7 Impact of hang time and crop level treatments on the yield and berry composition of Cabernet franc grapes 2011-2012.

Year	Factor	Yield/ vine	Clusters/ vine	Cluster weight (g)	Berry weight (g)	Vine size (kg)	Crop load	pH	Titrat-able acidity (g/L)	Brix	Anthoc-yanins (mg/L)	Color	Hue	Total phenols (mg/L)
2011	**Crop Level**													
	Full	2.8	29	99.1	1.23	0.71	4.4	3.59	5.35	25.1	449.9	0.562	0.714	1694.7
	Half	2.0	19	108.4	1.23	0.70	3.1	3.57	5.35	25.5	479.8	0.609	0.723	1776.8
	Significance[a]	****	****	*	ns	ns	****	ns	ns	*	ns	*	ns	ns
	Hang time													
	T0	2.8 a	24	117.4 a	1.34 a	0.68	4.5 a	3.53 b	5.30 b	23.6 c	505.0 a	0.618 a	0.696 b	1643.2 b
	T1	2.5 b	24	109.4 a	1.24 b	0.69	3.9 b	3.54 b	5.93 a	24.9 b	475.2 a	0.600 a	0.700 b	1678.8 b
	T2	1.9 c	24	83.9 b	1.12 c	0.73	2.8 c	3.68 a	4.81 c	27.4 a	414.3 b	0.538 b	0.759 a	1885.2 a
	Significance[a]	****	ns	****	****	ns	****	****	****	****	**	**	**	*
	Interaction	*	****	**	ns	*	***	ns	ns	ns	ns	ns	ns	ns
2012	**Crop Level**													
	Full	3.4	42	79.0	1.25	0.47	7.8	3.46	6.39	24.9	810.6	0.911	0.543	1932.1
	Half	2.4	26	90.7	1.20	0.49	5.5	3.46	6.31	25.1	855.4	0.932	0.539	1970.5
	Significance[a]	****	****	*	*	ns	****	ns	ns	ns	*	ns	ns	ns
	Hang time													
	T0	3.1 a	35	89.8 a	1.27 a	0.50	6.9 a	3.47 a	6.34 ab	23.4 c	877.6 a	0.994 a	0.515 c	1635.6 b
	T1	3.0 a	35	86.1 a	1.23 ab	0.46	7.3 a	3.47 a	6.28 b	25.2 b	878.1 a	0.892 b	0.530 b	2288.3 a
	T2	1.4 b	34	39.9 b	1.16 b	0.46	3.8 b	3.42 b	6.48 a	27.2 a	676.2 b	0.844 c	0.607 a	1923.3 ab
	Significance[a]	****	ns	****	*	ns	****	**	*	****	****	****	****	**
	Interaction	ns	ns	ns	*	*	ns	ns	ns	ns	ns	***	ns	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

Table 2.4.8 Impact of hang time and crop level treatments on the must composition of Cabernet franc 2011-2012.

Year	Factor	pH	Titratable acidity (g/L)	Brix	Antho-cyanins (mg/L)	Color	Hue	Total phenols (mg/L)
2011	**Crop Level**							
	Full	3.60	5.10	24.8	202.1	0.177	0.921	122.7
	Half	3.61	5.00	25.7	234.9	0.194	0.956	274.2
	Significance[a]	ns	ns	*	ns	*	ns	*
	Hang time							
	T0	3.40 c	5.85 a	22.8 b	272.0 a	0.159 b	0.572 c	140.9
	T1	3.53 b	5.85 a	26.4 a	293.0 a	0.176 b	0.820 b	257.6
	T2	3.89 a	3.35 b	26.6 a	91.0 b	0.222 a	1.424 a	197.0
	Significance[a]	****	****	*****	****	****	****	ns
	Interaction	ns	ns	ns	ns	ns	ns	ns
2012	**Crop Level**							
	Full	3.26	7.01	24.6	35.25	0.060	1.257	437.8
	Half	3.27	6.83	24.9	50.97	0.081	1.159	430.2
	Significance[a]	*	*	*	****	****	ns	ns
	Hang time							
	T0	3.21 c	6.96 a	23.1 c	25.74 b	0.047 b	1.196 a	326.9 b
	T1	3.27 b	7.20 a	24.9 b	25.56 b	0.046 b	1.271 a	471.2 ab
	T2	3.38 a	6.19 b	28.5 a	147.9 a	0.214 a	1.054 b	601.1 a
	Significance[a]	****	****	****	****	****	**	**
	Interaction	ns	ns	ns	****	****	****	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively.

Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

Table 2.4.9 Impact of hang time and crop level treatments on the wine composition of Cabernet franc 2011-2012.

Year	Factor	pH	Titratable acidity (g/L)	Ethanol (% v/v)	Anthocy-anins (mg/L)	Color	Hue	Total phenols (mg/L)
2011	**Crop Level**							
	Full	3.80	5.64	13.6	174.2	0.225	1.002	1280.8
	Half	3.81	5.75	14.0	169.6	0.264	0.985	1261.6
	Significance[a]	ns	ns	ns	ns	*	ns	ns
	Hang time							
	T0	3.51 c	6.16 a	12.7 b	242.9 a	0.297 a	0.760 b	1276.5 b
	T1	3.73 b	6.04 a	14.3 a	235.0 a	0.237 b	0.713 b	1891.7 a
	T2	4.18 a	4.89 b	14.3 a	37.78 b	0.199 b	1.508 a	645.5 c
	Significance[a]	****	****	***	****	**	****	****
	Interaction	ns	ns	ns	ns	ns	ns	ns
2012	**Crop Level**							
	Full	3.51	5.75	14.7	186.2	0.340	0.817	1378.8
	Half	3.50	6.01	15.1	213.8	0.349	0.758	1511.9
	Significance[a]	ns	ns	ns	ns	ns	*	ns
	Hang time							
	T0	3.41 b	5.92 b	13.9 c	254.1 a	0.322 b	0.629 b	1341.7
	T1	3.59 a	5.48 c	15.0 b	220.4 a	0.397 a	0.672 b	1541.7
	T2	3.52 a	6.44 a	16.3 a	88.15 b	0.300 b	1.196 a	1456.3
	Significance[a]	***	**	**	****	***	****	ns
	Interaction	ns	ns	ns	ns	ns	*	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

Table 2.4.10 Impact of hang time and crop level treatments on the yield and berry composition of Cabernet Sauvignon 2011-2012.

Year	Factor	Yield /vine	Clusters /vine	Cluster weight (g)	Berry weight (g)	Vine size /vine	Crop load	pH	Titratable acidity (g/L)	Brix	Antho-cyanins (mg/L)	Color	Hue	Total phenols (mg/L)
2011	**Crop Level**													
	Full	2.5	30	85	1.13	0.62	5.0	3.50	6.28	24.4	497.4	0.637	0.733	1806.8
	Half	1.8	23	78	1.11	0.56	4.0	3.51	6.23	24.4	540.9	0.664	0.719	1806.6
	Significance[a]	****	****	**	ns	ns	**	ns	ns	ns	*	ns	ns	ns
	Hang time													
	T0	2.4 a	26 ab	91.9 a	1.22 a	0.56	5.2 a	3.50 b	6.11 b	22.6 c	584.1 a	0.678 a	0.715	1811.4 ab
	T1	2.3 a	28 a	81.1 b	1.16 b	0.61	4.5 ab	3.46 b	6.57 a	24.1 b	496.3 b	0.661 a	0.743	1945.5 a
	T2	1.8 b	25 b	71.4 c	0.99 c	0.59	3.9 b	3.57 a	6.08 b	26.5 a	477.1 b	0.612 b	0.72	1663.3 b
	Significance[a]	****	*	****	****	ns	**	****	****	****	****	**	ns	**
	Interaction	ns	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2012	**Crop Level**													
	Full	2.9	33	87.0	1.13	0.49	9.3	3.40	6.74	25.5	1076.9	1.214	0.500	2614.5
	Half	1.6	22	75.9	1.10	0.43	5.9	3.42	6.6	25.9	1083.9	1.308	0.496	2627.9
	Significance[a]	****	****	**	ns	ns	***	ns	ns	ns	ns	**	**	ns
	Hang time													
	T0	2.4	29	83.6	1.20 a	0.44	9.0	3.39 b	6.46 c	24.4 c	1127.5 a	1.267	0.479 b	2691.7 a
	T1	2.0	26	79.4	1.11 b	0.48	6.2	3.44 a	6.73 b	26.3 b	1076.3 a	1.265	0.508 a	2760.4 a
	T2	----	----	----	0.86 c	----	----	3.39 b	7.12 a	28.2 a	951.4 b	1.232	0.519 a	2118.0 b
	Significance[a]	ns	ns	ns	****	ns	ns	*	****	****	*	ns	****	ns
	Interaction	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	*	****	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test

Table 2.4.11 Impact of hang time and crop level treatments on the must composition of Cabernet Sauvignon 2011-2012.

Year	Factor	pH	Titratable acidity (g/L)	Brix	Anthocyanins (mg/L)	Color	Hue	Total phenols (mg/L)
2011	**Crop Level**							
	Full	3.51	6.09	23.8	326.4	0.188	0.653	1017.7
	Half	3.52	6.02	23.9	317.2	0.192	0.649	949.5
	Significance[a]	ns	ns	ns	ns	ns	ns	ns
	Hang time							
	T0	3.35 c	6.96 a	21.9 c	347.2 a	0.185	0.453 b	875.8 b
	T1	3.45 b	6.90 a	24.4 b	415.3 a	0.202	0.555 b	1170.5 a
	T2	3.75 a	4.32 b	25.2 a	202.9 b	0.183	0.944 a	904.6 b
	Significance[a]	****	****	****	***	ns	****	**
	Interaction	ns	ns	ns	ns	ns	ns	ns
2012	**Crop Level**							
	Full	3.22	7.71	24.5	111.6	0.124	0.834	854.6
	Half	3.26	7.26	25.1	94.2	0.125	0.851	921.9
	Significance[a]	**	**	**	****	ns	ns	ns
	Hang time							
	T0	3.16 c	8.46 a	23.2 c	38.81 c	0.048 c	0.902 a	866.7 b
	T1	3.28 b	7.05 b	25.1 b	101.0 b	0.107 b	0.807 b	815.9 b
	T2	3.35 a	5.85 c	29.1 a	301.0 a	0.409 a	0.771 b	1169.9 a
	Significance[a]	****	****	****	****	****	****	****
	Interaction	ns	ns	***	****	ns	*	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

Table 2.4.12 Impact of hang time and crop level treatments on the wine composition of Cabernet Sauvignon 2011-2012.

Year	Factor	pH	Titrat-able acidity (g/L)	Ethanol (% v/v)	Anthocyanins (mg/L)	Color	Hue	Total phenols (mg/L)
2011	**Crop Level**							
	Full	3.66	6.01	12.4	295.5	0.374	0.825	1648.0
	Half	3.68	5.99	12.3	276.8	0.362	0.894	1612.1
	Significance[a]	ns	ns	ns	ns	ns	ns	ns
	Hang time							
	T0	3.45 c	6.44 a	11.5 b	348.5 a	0.449 a	0.649 b	1665.9 b
	T1	3.59 b	6.22 a	12.4 ab	392.9 a	0.397 b	0.687 b	2131.1 a
	T2	3.98 a	5.35 b	13.2 a	117.0 b	0.258 c	1.242 a	1093.2 c
	Significance[a]	****	****	*	****	****	****	****
	Interaction	ns	ns	ns	ns	ns	ns	ns
2012	**Crop Level**							
	Full	3.36	6.51	14.8	427.3	0.647	0.606	2156.9
	Half	3.47	6.61	14.7	403.6	0.600	0.656	2018.8
	Significance[a]	**	ns	ns	ns	*	***	ns
	Hang time							
	T0	3.42 ab	6.30 b	14.2	443.1 b	0.637 b	0.583 b	2150.0 a
	T1	3.36 b	6.49 b	15.4	553.2 a	0.727 a	0.573 b	2333.3 a
	T2	3.48 a	7.04 a	14.6	167.4 c	0.448 c	0.791 a	1626.3 b
	Significance[a]	ns	*	ns	****	***	****	**
	Interaction	**	ns	ns	*	***	***	**

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

Table 2.4.13 Pinot gris, interactive results for yield/berry analysis.

Year	Crop level	Hang level	Yield (kg/vine)	Clusters/ vine	Cluster wt. (g)	Vine size (kg/vine)	Ravaz index	pH	Titratable acidity (g/L)	Brix	Berry weight (g)
2011	HC	T0	2.4	21	116.8	0.52	5.8	3.22	8.125	19.82	1.38
	HC	T1	1.9	22	86.6	0.49	4.6	3.27	8.613	19.59	1.39
	HC	T2	1.6	22	75.1	0.5	3.7	3.19	8.452	21.50	1.23
	FC	T0	3.5	29	119.3	0.51	8.4	3.24	8.293	19.62	1.42
	FC	T1	2.6	32	81.4	0.5	6.1	3.24	8.550	18.98	1.43
	FC	T2	2.3	32	70.6	0.59	4.9	3.23	8.358	21.13	1.24
Year	Crop level	Hang level	Yield (kg/vine)	Clusters/ vine	Cluster wt. (g)	Vine size (kg/vine)	Ravaz index	pH	Titratable acidity (g/L)	Brix	Berry weight (g)
2012	HC	T0	3.9	30	133.1	0.42	12.7	3.48	5.368	21.23	1.26
	HC	T1	2.8	28	104.6	0.41	8.9	3.57	6.278	23.66	1.23
	HC	T2	2.7	28	97.9	0.37	8.6	3.56	6.024	26.12	1.16
	FC	T0	5.9	43	142.7	0.39	17.2	3.5	5.386	19.82	1.32
	FC	T1	4.5	44	100.8	0.41	12.9	3.52	6.524	22.45	1.24
	FC	T2	3.7	42	88	0.46	10.2	3.57	6.063	25.63	1.23

Table 2.4.14 Riesling, interactive results for yield/berry analysis.

Year	Crop level	Hang level	Yield (kg/vine)	Clusters/ vine	Cluster weight (g)	Vine size (kg/vine)	Ravaz index	pH	Titratable acidity (g/L)	Brix	Berry weight (g)
2011	HC	T0	2.2	25	89.2	0.44	5.4	3.22	8.13	19.8	1.38
	HC	T1	1.9	26	76.6	0.4	6.2	3.27	8.61	19.6	1.39
	HC	T2	1.1	19	57.5	0.4	3.1	3.19	8.45	21.5	1.23
	FC	T0	4	43	93.7	0.46	9.1	3.24	8.29	19.6	1.42
	FC	T1	3	38	78.7	0.4	9.9	3.24	8.55	18.9	1.43
	FC	T2	1.9	31	61.8	0.45	5.2	3.24	8.36	21.1	1.24
Year	Crop level	Hang level	Yield (Kg/vine)	Clusters/ vine	Cluster weight (g)	Vine size (Kg/vine)	Ravaz index	pH	Titratable acidity (g/L)	Brix	Berry weight (g)
2012	HC	T0	2.6	25	105.7	0.36	8.2	3.33	7.05	20	1.59
	HC	T1	2.7	23	115	0.35	10.2	3.38	6.93	20.7	1.58
	HC	T2	1.4	18	74.4	0.37	4.3	3.29	7.95	22.4	1.17
	FC	T0	4.7	41	118.2	0.36	13.6	3.31	7.71	18.8	1.51
	FC	T1	4.6	40	115.1	0.36	13.5	3.33	7.27	19.5	1.56
	FC	T2	2.6	33	80.3	0.39	8.0	3.3	8.2	20.7	1.39

Table 2.4.15 Cabernet franc, interactive results for yield/berry analysis.

Year	Crop level	Hang level	Yield (kg/vine)	Clusters/ vine	Cluster weight (g)	Vine size (kg/vine)	Ravaz index	pH	Titratable acidity (g/L)	Brix	Berry weight (g)	Anthocyanins (mg/L)	Color	Hue	Total phenols (mg/L)
2011	HC	T0	2.3	18	122.8	0.72	3.3	3.51	5.4	23.7	1.35	516	0.653	0.705	1620
	HC	T1	2.1	18	120.9	0.66	3.4	3.53	5.96	24.9	1.23	490	0.603	0.706	1748
	HC	T2	1.7	22	80	0.71	2.5	3.67	4.68	27.8	1.11	433	0.571	0.757	1962
	FC	T0	3.4	30	111.8	0.64	5.8	3.55	5.2	23.6	1.32	494	0.583	0.687	1667
	FC	T1	2.9	31	97.3	0.73	4.3	3.55	5.91	24.8	1.25	460	0.597	0.695	1609
	FC	T2	2.2	25	88	0.75	3.1	3.68	4.94	27	1.13	395	0.505	0.760	1808
Year	Crop level	Hang level	Yield (kg/vine)	Clusters/ vine	Cluster weight (g)	Vine size (kg/vine)	Ravaz index	pH	Titratable acidity (g/L)	Brix	Berry weight (g)	Anthocyanins (mg/L)	Color	Hue	Total phenols (mg/L)
2012	HC	T0	2.5	26	95.1	0.54	5.0	3.46	6.31	23.5	1.26	902	0.951	0.517	1701
	HC	T1	2.3	27	86.5	0.44	5.9	3.47	6.25	25.4	1.24	901	0.927	0.526	2328
	HC	T2	3.7	44	84.9	0.47	8.7	3.44	6.42	27.6	1.08	671	0.906	0.613	1854
	FC	T0	3.6	43	85.9	0.48	8.6	3.48	6.38	23.3	1.29	853	1.038	0.514	1570
	FC	T1	1.4	34	39.9	0.46	3.8	3.47	6.31	25.1	1.22	855	0.858	0.534	2249
	FC	T2	-	-	-	-	-	3.4	6.53	26.9	1.24	680	0.798	0.602	1986

Table 2.4.16 Cabernet Sauvignon, interactive results for yield/berry analysis.

Year	Crop level	Hang level	Yield (kg/vine)	Clusters/ vine	Cluster weight (g)	Vine size (kg/vine)	Ravaz index	pH	Titratable acidity (g/L)	Brix	Berry weight (g)	Anthocyanins (mg/L)	Color	Hue	Total phenols (mg/L)
2011	HC	T0	1.93	21	91.6	0.51	4.7	3.5	6.01	22.6	1.2	606.2	0.705	0.708	1873
	HC	T1	1.95	25	79	0.65	4.2	3.47	6.66	24	1.15	529.8	0.663	0.726	1898
	HC	T2	1.47	23	63.3	0.52	3.3	3.57	6.03	26.5	0.99	486.5	0.626	0.725	1648
	FC	T0	2.89	31	92.3	0.61	5.8	3.49	6.22	22.6	1.24	562.0	0.652	0.723	1750
	FC	T1	2.54	31	83.2	0.58	4.7	3.46	6.48	24.1	1.17	462.7	0.660	0.761	1992
	FC	T2	2.08	27	79.6	0.66	4.4	3.56	6.13	26.6	0.99	467.6	0.598	0.715	1678
Year	Crop level	Hang level	Yield (kg/vine)	Clusters/ vine	Cluster weight (g)	Vine size (kg/vine)	Ravaz index	pH	Titratable acidity (g/L)	Brix	Berry weight (g)	Anthocyanins (mg/L)	Color	Hue	Total phenols (mg/L)
2012	HC	T0	1.72	23	77.1	0.39	6.8	3.41	6.26	24.8	1.16	1116.5	1.279	0.483	2674
	HC	T1	1.47	20	74.7	0.47	5.0	3.44	6.75	26.4	1.11	1080.0	1.307	0.512	2818
	HC	T2	3.19	35	90.2	0.49	11.3	3.41	7.2	28	0.88	997.5	1.402	0.487	2060
	FC	T0	2.55	30	84	0.49	7.4	3.37	6.66	24	1.24	1138.5	1.256	0.477	2709
	FC	T1	-	-	-	-	-	3.43	6.72	26.2	1.12	1072.5	1.224	0.505	2703
	FC	T2	-	-	-	-	-	3.37	7.05	28.3	0.82	905.4	1.064	0.553	2176

Table 2.5 Harvest days for 2011 and 2012 with commercial harvest as T0

Cultivar	Hang time	Date (2011)	Date (2012)
Pinot gris	T0	22-Sep-11	11-Sep-12
	T1	11-Oct-11	02-Oct-12
	T2	01-Nov-11	25-Oct-12
Riesling	T0	11-Oct-11	25-Sep-12
	T1	01-Nov-11	16-Oct-12
	T2	22-Nov-11	08-Nov-12
Cabernet Franc	T0	22-Oct-11	02-Oct-12
	T1	07-Nov-11	23-Oct-12
	T2	06-Dec-11	15-Nov-12
Cabernet Sauvignon	T0	22-Oct-11	16-Oct-12
	T1	07-Nov-11	08-Nov-12
	T2	06-Dec-11	27-Nov-12

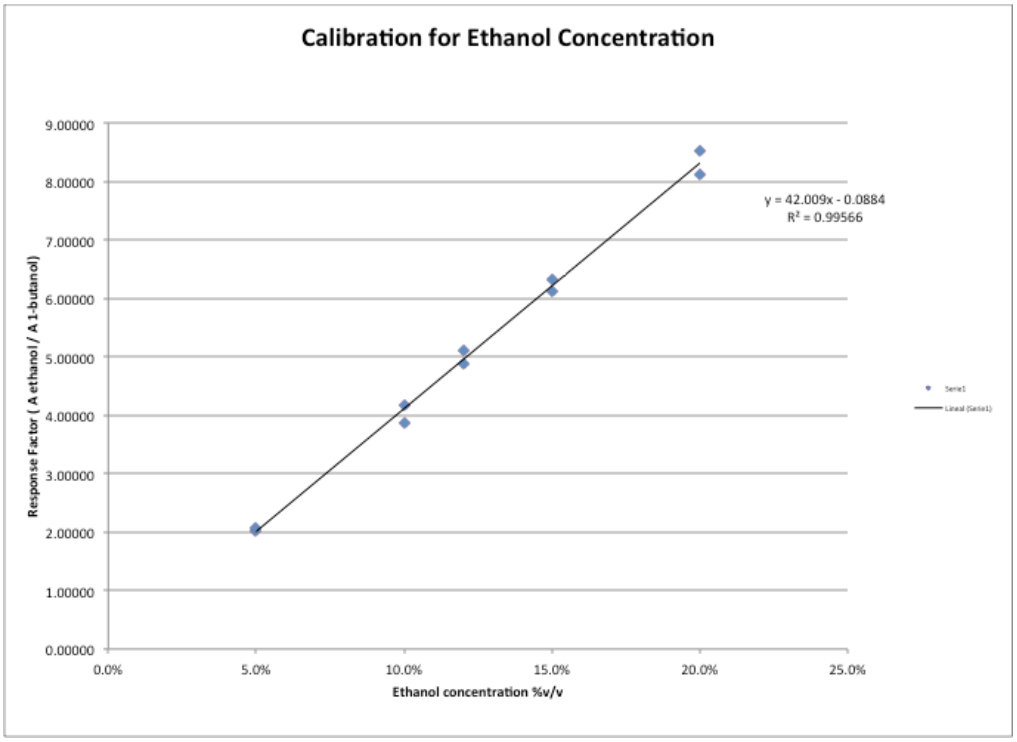

Figure 2.1 Calibration curve for ethanol quantification in wine

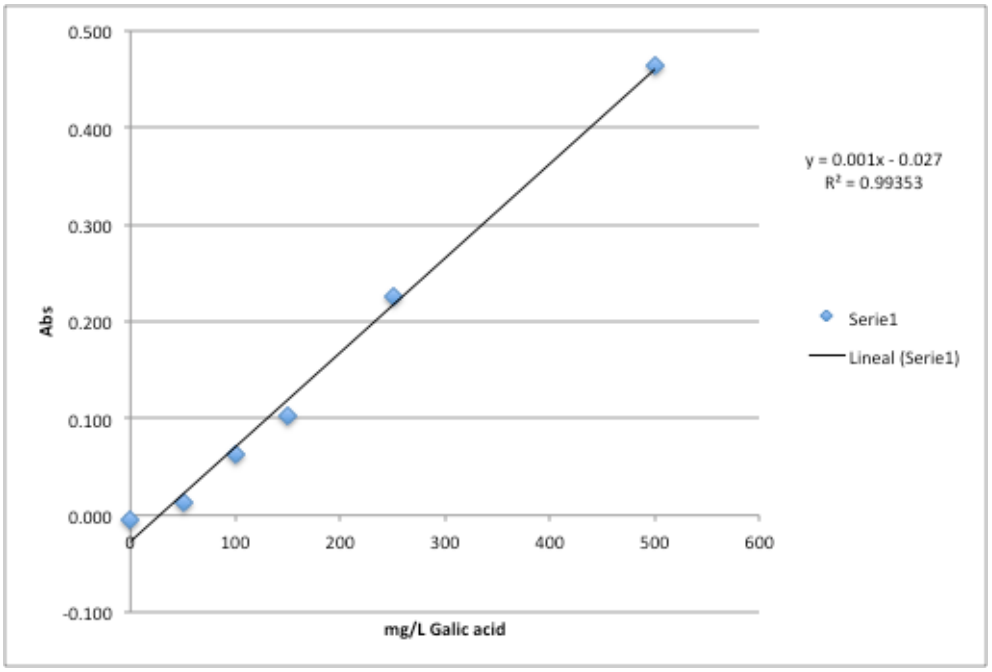

Figure 2.2 Calibration curve for total phenols in grape berries, must and wine from Cabernet franc and Sauvignon. Results expressed as equivalents of Gallic acid.

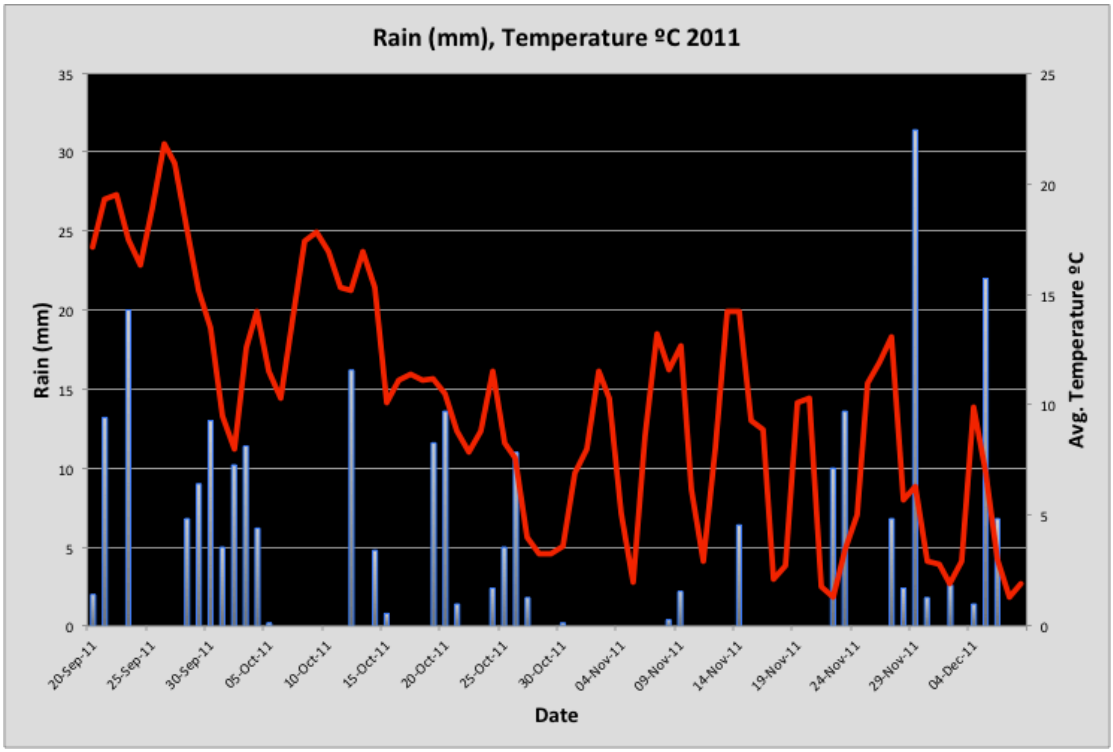

Figure 2.3 Main daily rainfall (mm/day) and temperature (ºC) during harvest period year 2011 at NOTL Virgil station, ON.

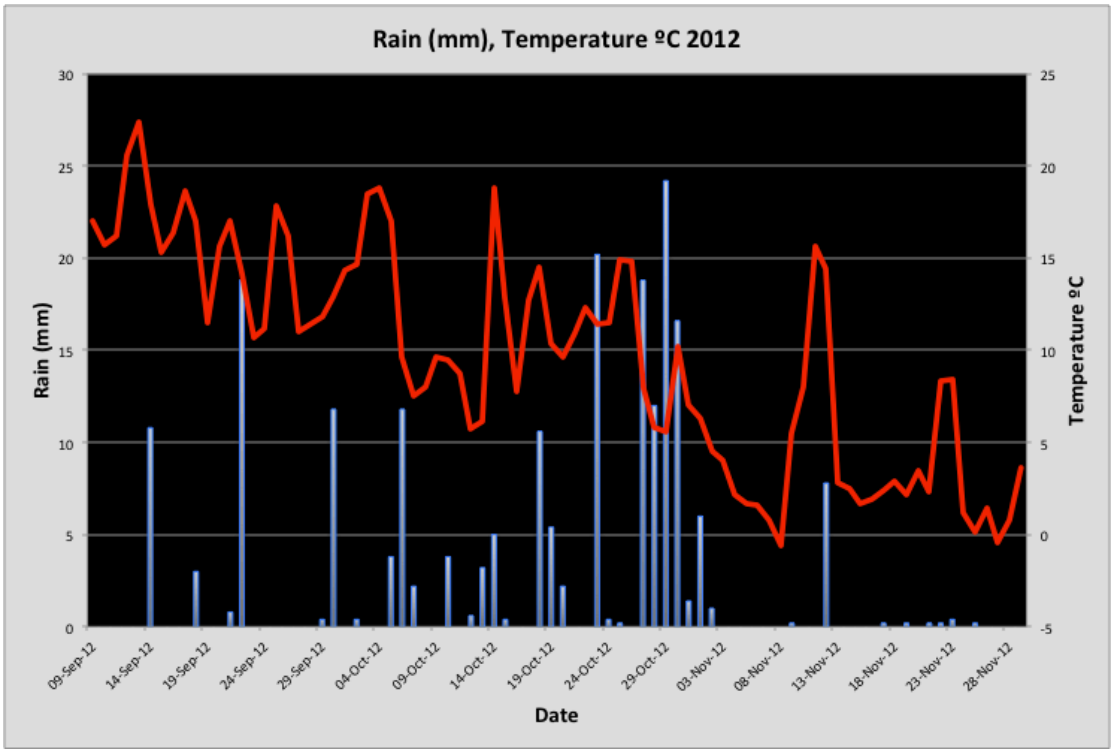

Figure 2.4 Main daily rainfall (mm/day) and temperature (ºC) during harvest period year 2012 at NOTL Virgil station, ON.

CHAPTER 3

IMPACT OF CROP LEVEL AND HANG TIME ON THE AROMA OF FOUR WINE GRAPE CULTIVARS FROM THE NIAGARA REGION

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3.1 Abstract: Pinot gris, Riesling, Cabernet franc and Cabernet Sauvignon vines from a single vineyard in Niagara-on-the-Lake, Ontario, Canada, were subjected to two viticultural treatments during 2011 and 2012 vintages in a randomized experiment. The treatments were, reduction in crop to one cluster (basal) per shoot, (half crop, HC), was imposed at veraison plus any reduction in crop, with a control without reduction (full crop, FC) followed by three harvest dates; T0 at commercial harvest, three weeks after T0 (T1), and six weeks after T0 (T2), all with subsequent wine production. It was hypothesized that keeping a full crop with a longer hang time might have a greater impact on wine aroma than to reduce the crop level. Analysis of wine aroma was carried out by gas chromatography mass spectrometry (GC-MS). Selected aroma compound were quantified by calibration using analytical standards prepared at different concentrations in model wine. Generally delay in harvest overcame the effect of crop reduction in almost all components. Some cultivars benefitted with an increase in varietal aromas like monoterpenes in white cultivars or the increase in some esters like ethyl caprylate. A reduction in concentration due to increased hang time was also evident and beneficial in odour quality, e.g. the reduction of volatile acids, reduction in grassy-green like odours in wine. Even though increase in ethanol was related to the increase of sugars in grape and must, the relationship with higher alcohols were less apparent. The increase in hang time in some cases was disadvantageous, particularly at T2. Production at different levels of compounds like benzaldehyde, diethyl acetal, or higher concentrations of high alcohols like isoamyl alcohol or nonanol could be linked to spoilage of grapes before harvest.

Key words: Crop reduction, Hang time, vintage effect, gas chromatography mass spectrometry, wine aroma composition.

3.2 Introduction

In wine, > 800 compounds have been identified in their volatile fraction (Ortega et al. 2002). Some of these compounds can be associated with varietal characteristics or are generated during fermentation, while others are considered undesirable when they occur (Bakker and Clarke, 2012). Volatile compounds become part of the wine mix by different sources, e.g. grape sugars, where fermentation releases as prime metabolites ethanol and CO_2, and secondary metabolites like esters, acids and higher alcohols: non-volatile grape-derived precursors that could be release by enzymatic action by bacteria and yeast like monoterpenes, norisoprenoids and some thiols, and secondary metabolites from the action of malo-lactic bacteria resulting in some esters and diacetyl (Borneman et al. 2012).

The effects of crop level reduction are generalized with an increase in sugars in grapes (Freeman and Kliewer 1983; Jackson and Lombard 1993; Reynolds et al. 1994), with a concomitant increase of ethanol in wine produced (Reynolds et al. 1996). The effect of crop reduction increased the concentration of free and potential terpenes (Reynolds and Wardle 1989; Reynolds et al. 2007), increased arginine and proline (Freeman and Kliewer 1983; Kliewer and Ough 1970), amino acids responsible for increase in the fermentation rate and producing a more intensive aroma (Bell et al. 1979), as well as increase of volatile acids (Bravdo et al. 1984).

Delay of harvest is linked also to an increase in sugar concentration by a reduction in berry weight due to dehydration processes (Chkaiban et al. 2007; Constantini et al. 2006). In addition to sugar concentration, phenolics and aroma compounds are either concentrated or produced (Bellincontron et al. 2004; Constantini et al. 2006). The drying of fruit also generates shrinkage, which modifies the shape and dimension of products affecting the mass transport phenomena (Kays 1997; Wang and Brennan 1995). With a loss of water, the cell wall enzyme activity is increased, and accelerates respiration and ethylene production. This change or reduction of volatiles and polyphenols is not only due to concentration but to changes in metabolism (Bellincontron et al. 2004; Constantini et al. 2006; Zamboni et al. 2008).

Dehydration by controlled processes reduced ethyl acetate and acetic acid (Moreno et al. 2008), increased ethanol and acetaldehyde, with reduction

of alcohol dehydrogenase and proline (Zamboni et al. 2008). Wines made from dehydrated grapes contained more terpenes and norisoprenoids (Moreno et al. 2008), but by the dehydration process a decline in carotenoid profiles was apparent (Chkaiban et al. 2007). Grapes imposed to process of dehydration are susceptible to microorganism spoilage like *Botrytis cinerea*-derived increases in polyalcohols, and production in high amounts of other high alcohols like glycerol, arabitol and mannitol (Sponholz 1988). Sour rot reduced the free geraniol, nerol and linalool concentrations and increased trans-furan linalool oxide, benzyl alcohol, 2-phenetyl ethanol, 2-methy-1-propanol and 3-methyl-1-butanol in Riesling (Zoecklein et al. 2010).

It was chosen to experiment with different "hang times" (harvest dates) to determine whether keeping a full crop with a more lengthy hang time might have a greater impact on wine volatile composition than to reduce the crop level. The overall objective for this project was to determine the impact of hang time and crop control on the wine volatile composition of four grape cultivars; two whites (Riesling and Pinot gris), and two reds (Cabernet Sauvignon and Cabernet franc); commonly produced in the Niagara Peninsula, Ontario, Canada.

3.3 Materials and Methods

3.3.1 Experimental design

Two crop levels were imposed at veraison, half crop and full crop, and three harvest dates (including two hang times after regular harvest) were combined in a factorial treatment arrangement containing six treatment combinations. During two years of harvest, 2011, 2012, analysis of volatile compounds in wine samples by gas chromatography-mass spectrometry (GC-MS) was performed to determine whether differences existed between the two crop levels and between the hang times. Analysis of volatile compounds was also performance in Riesling must sample 2012.

3.3.2 Sample preparation

The analysis of volatile compounds in wine was based on Bowen and Reynolds (2012) with adjustments. Aroma analysis by GC-MS was carried out in final wines for 2011 and 2012 for the four grape vine cultivars. A sample of 30 mL was taken from each wine and each treatment as was described in Chapter 2 (see section 2.3.4), and were kept at 4 ºC in presence of N_2 inert

gas until analysis. In duplicate, 100 μL of an internal standard, prepared with 10 μL of 1-dodecanol 98% (Aldrich; Oakville, ON) in 10 mL of ethyl alcohol 100% (Commercial Alcohols; Brampton, ON), was poured in 10-mL volumetric flask followed with the addition of wine to the mark and mixed. The prepared sample was transferred into a 10 mL Gerstel extraction vial. A 10-mm stir bar commercially known as Twister (Gerstel, Baltimore, MD) coated with polydimethylsiloxane (PDMS; 0.5 mm film thickness) was added to the sample and stirred for 1 hr. at 1000 rpm for extraction at room temperature. After extraction the stir bar was removed, rinsed with Milli-Q water (Millipore, Bedford, MA) and dried out with lint free tissue, then placed in a 4-mL amber vial at 4 ºC until analysis during the same day. The stir bar was then inserted inside extraction glass tube inside the "Termal desorption unit", TDU, attached to GC-MS instrument.

3.3.3 GC-MS conditions

An Agilent 6890N/5975B GC-MS equipped with a Gerstel thermal desorption unit (TDS), cooled injection system (CIS) and programmable temperature vaporization (PTV) was used for the analysis of aromas of wine in 2011 and 2012. One capillary column Agilent 19091S-433 HP-5MS 5% phenyl methyl siloxane, nominal length 30.0 m, nominal diameter 250.00 um, nominal film thickness 0.25 um; and one capillary column J&W 122-7032 DB-WAX nominal length 30.0 m, nominal diameter 250.00 um, nominal film thickness 0.25 um were used for the analysis with these instrument conditions:

- Gerstel TDS. Initial temperature: 30 ºC, ramp rate 1 at 60 ºC/min. End temperature at 250 ºC, hold time 5.00 min, transfer temp 275 ºC. Desorption mode: Splitless.
- Gerstel CIS. Initial temperature -120 ºC, equilibration time 0.25 min, initial time 0.20 min, ramp rate 12.0 ºC/s. End temperature 280 °C, cryo-cooling used.
- GC-oven conditions: Initial temp: 35 ºC, He carrier (1.4 mL/min).

Rate	Final temp	Final time
4ºC/min	155ºC	0
3ºC/min	240ºC	5 min

MS information: Solvent delay: 3 min, SCAN acquisition method for identification compounds, low mass: 30, high mass: 400, threshold: 150, and SIM/SCAN mode for quantification of aromatic compounds.

3.3.4 Conditioning of material

Stir bars used for extraction were previously conditioned every time before use to avoid any cross contamination. After analysis each stir bar was overnight in a solution of 80:20 acetonitrile/methanol respectively, let dry and then placed it at 250 ºC for 2 hr. with a constant flow of N_2 inert gas. All glass material was washed with Milli-Q water (Millipore, Bedford, MA) and methanol and then dried out at 250 ºC for 1 hr.

3.3.5 Calibration compounds and odour active values

Scan analysis reflected the presence of more than 100 volatile compounds in wines from all cultivars. For calibration purposes, 30 compounds were chosen as the highest in quality and presence to obtain their concentration. Three point calibration curves were created for each compound for quantification. Aromatic standards were obtained from: Fisher Scientific: Ethyl acetate and α-terpineol; Sigma-Aldrich: Isobutyl alcohol, 1-hexanol, decanal, Damascenone, Isoamyl alcohol and 1-octanol; Aldrich: Ethyl butyrate, Isoamyl acetate, Hexyl acetate, Benzaldehyde, Ethyl heptanoate, Octanoic acid, Diethyl acetal, Ethyl caproate, Hexanoic acid, Ethyl phenyl acetate and Ethyl decanoate; Fluka: Terpinolene, 1-nonanol, Diethyl succinate, Linalool and 2-phenetyl acetate; Acros organics: Ethyl caprylate, Phenyl ethyl alcohol, 1-heptanol, β-Citronellol and Geraniol; Sigma: Decanoic acid.

Model wine was use for calibration curves and prepared based on Zacalain et al. (2007), using 12% (v/v) of pure anhydrous ethyl alcohol (Commercial Alcohol, Brampton, ON) diluted in Milli-Q water (Millipore, Bedford, MA) and 5 g/L of tartaric acid. The pH of model wine was adjusted to 3.6 with 1M NaOH. Each aroma standard was diluted first in pure anhydrous ethanol at 1000 mg/L and kept at 4 ºC until analysis, then diluted at different concentrations in synthetic wine. Calibration samples were analyzed in SIM/SCAN mode using same condition as described in 3.3.2 with the use of the same internal standard. Odour active values (OAVs) were calculated as a

ratio between the each concentrations obtained by calibration versus their respective threshold. The thresholds for this experiment were obtained from literature (table 3.4.11.). OAV are shown in table 3.4.10.

3.3.6 Analysis of Terpenes in Riesling must 2012

The analysis of must was separated in two specific treatments base on the work of Moio et al. (2004) the first, without the β-glucosidase solution to determine the free-terpene, adding 1.5 mL of distilled water; and the second, using the β-glucosidase solution, to obtain the bound-terpene linked to the sugar. To prepare the enzymatic reaction the must samples were centrifuged using an IEC centrifuge (Model CL2, Thermo Scientific, Waltham, MA), during 10 min at 4500 rpm. The supernatant was recovered and the pH adjusted around 5.0 with NaOH at 20% (Sigma-Aldrich). An enzymatic solution of commercial β-glycosidases LAFAZYM®AROM (Laffort, Sacramento, CA) was prepared using 1.5 mg in 7.5 mL of distilled water, 1.5 mL from this solution was mixed after with the must previously described. The mix was incubated at 40°C during 12 hours in constant agitation to allow reaction. During the sample preparation, N_2 inert gas was added at different steps to avoid oxidation of volatile terpenes present in the must. For both treatments, 10 mL of prepared sample was analyzed in GC-MS by duplicate in the same way as described in 3.3.2.

3.3.7 Statistical analysis

All concentration obtained from calibration curves were analyzed with SAS analytics statistics software for analysis of variance to determine whether effects could exist between crop levels treatments and hang time. Duncan´s multiple range test, was used to compare levels of group means (Lea et al. 1997)

3.4 Results

Two sets of results are shown in this section; the first one (Tables 3.4.1 – 3.4.4) represents all the statistical data for the volatile compounds analysis for crop and hang time, plus the statistical analysis for the terpene concentration in Riesling must samples for 2012 (Table 3.4.5), the second contains OAVs found in wine samples for 2011 and 2012 (Table 3.4.10). Table 3.4.11 shows each volatile standard with retention times (RT),

calibration intervals, r^2 values, and odour quality used for quantification. Differences in vintage were discussed before (Chapter 2.5).

3.4.1 Pinot gris

Pinot gris had contained 23 volatile compounds. Some were highly odour active (Table 3.4.10) and could generate an impact over the general aroma in the wine**.**

Statistical analysis for wine volatile compounds (Table 3.4.1). The statistical analysis for 2011 and 2012 wines showed a few differences between treatments at each volatile compound. With respect to crop differences, just isobutyl alcohol, diethyl succinate (only detected in half crop treatment) and terpinolene had differences between half and full crop in 2011. An important difference here is that isobutyl alcohol was not detected in wines from 2012 as well as citronellol. For this year 1-hexanol, hexyl acetate, ethyl phenyl acetate and terpinolene were reduced in concentration with reduction of crop, while ethyl acetate and damascenone increased.

With respect of hang time, more compounds had greater impact in time than crop reduction; 1-hexanol increased in time but only in 2012. The concentrations in this years were lower than 2011. Isobutyl alcohol was reduced in concentration in 2011 but no presence was detected in 2012. Hexyl acetate was reduced in concentration in both years finishing with undetected levels at T2. Octanoic acid was reduced in concentration in 2011, but increased slightly in 2012. Diethyl acetate was only detected at T2 in both years. Similar behaviour occurred with citronellol in 2011, but it was not present in 2012. Ethyl caproate was reduced in concentration in 2011 relative to extended hang time, and increased in 2012, and for this compounds a higher concentration was detected in all harvest dates compared with previous year. Ethyl phenyl acetate increased in concentration in both years relative to harvest date while diethyl succinate was reduced in 2011 and increased in 2012. Terpinolene decreased with extended hang time in 2011 but was just detected in T2 in 2012, while decanoic acid in both years showed a decrease with increased hang time.

3.4.2 Riesling

Riesling contained 27 volatile compounds. Some were highly odour active (Table 3.4.10) and could generate an impact over the general aroma in the wine**.**

Statistical analysis for volatile compounds (Table 3.4.2). Reduction in crop affected a few compounds between years. Ethyl butyrate, phenyl ethyl alcohol, citronellol, geraniol and damascenone were reduced by cluster thinning in 2012 but were without impact in 2011. 1-Hexanol and isobutyl alcohol were reduced in concentration in both years with reduction of crop, the last was not detected in the half crop treatment in 2012. Some compounds were just detected in one year. For 2011, diethyl acetal was only present in FC, geraniol increased with crop reduction, and terpineol was not impacted. Hexyl acetate had an increase in 2011 with crop reduction but was reduced in 2012, and the same behaviour for isoamyl acetate and terpinolene was observed. Ethyl phenyl acetate had a reduction in cluster thinned wines in 2011 but was only detected when crop was reduced in 2012.

Increase in hang time led to an increase in concentration for ethyl butyrate, isobutyl alcohol, terpinolene and citronellol, and a decrease for hexyl acetate, octanoic acid, hexanoic acid, ethyl caprylate and damascenone in both years. 1-Hexanol and isoamyl acetate decrease in 2011 but increased in 2012 while ethyl acetate and decanoic acid observed the opposite trend. Some compounds were just present in one or two harvest dates in one of both years. Diethyl acetal was only present in T2/FC in 2011, 1-heptanol in T2/FC in 2012, geraniol present with a decrease in all hang levels but just in 2011, and benzaldehyde only present in 2012 in both crop levels at the T2.

3.4.3 Cabernet franc

Cabernet franc contained 22 volatile compounds. Some were highly odor active (Table 3.4.10) and could generate an impact over the general aroma in the wine**.**

Statistical analysis for volatile compounds (Table 3.4.3). Reductions in crop led to increases in concentration in citronellol and ethyl phenyl acetate and reductions in ethyl heptanoate in both years. In 2012, there were crop reduction-related increases in concentration present for 1-hexanol, ethyl caprylate, ethyl caproate, 2-phenetyl acetate, isoamyl acetate,

ethyl acetate and Benzaldehyde, while reductions also occurred in diethyl acetal and diethyl succinate. 1-Heptanol and decanal were detected in 2012 only, with a reduction and increase in concentration, respectively, relative to crop reduction. Ethyl butyrate was the only compound present in both years that increased with crop reduction in 2011.

With respect to hang time, in both years, diethyl acetal and diethyl succinate increased with increased hang time, while 1-hexanol, citronellol, damascenone and decanoic acid reduced. Compounds affected in 2011 relative to extended hang time were ethyl butyrate, with an increase in concentration and isobutyl alcohol, phenyl ethyl alcohol, octanoic acid and isoamyl alcohol with reductions. In 2012, ethyl caprylate and ethyl phenyl acetate increased relative to hang time, but the later was not detected at T1. Both 1-heptanol and decanal were only present 2012 the first at both crop levels and the second just in samples of FC/T1. Ethyl heptanoate and benzaldehyde were only present in at the last hang time in both years.

3.4.4 Cabernet Sauvignon

Cabernet Sauvignon contained 21 volatile compounds; some were highly odour active (Table 3.4.10) and could generate an impact over the general aroma in the wine**.**

Statistical analysis for volatile compounds (Table 3.4.4). Reduction in crop led to a reduction in concentration for 1-hexanol and increase for benzaldehyde in both years. Compounds that were affected by crop reduction with reduction in concentration were phenyl ethyl alcohol, octanoic acid, isoamyl alcohol, ethyl caproate, 2-phenetyl acetate and decanoic acid just in 2011. Diethyl acetal in 2012 was reduced with reduction in crop while diethyl succinate and ethyl heptanoate increased. Hexanoic acid was only present in HC/T0 in 2011 while it was reduced in concentration with crop reduction in 2012. 1-nonanol on the other hand was just present in HC/T2 in 2012 but was reduced in concentration with reduction in crop in 2011.

Increased hang time led to a reduction in concentrations in 1-hexanol, phenyl ethyl alcohol, hexanoic acid and decanoic acid in both years. Ethyl butyrate reduced in 2012 but was only present at T2 in 2011. Ethyl caproate followed the same trend but was present in all hang times in 2011. Octanoic acid was only reduced in 2011. Isobutyl alcohol and citronellol were only

detected in 2012 samples with reduction. The latter was not present in T2. Ethyl heptanoate was also just present in 2012 but with an increase between T1 and T2. Diethyl acetate was just present just in the first hang time (T0) in 2011, while just present in FC/T2 in 2012. Benzaldehyde was only present at T2 in both years; the difference is that it was only detected in reduced crop in 2011. Damascenone was only detected in 2011 samples with a reduction in concentration with increasing hang time.

3.5 Discussion

As mentioned in Chapter 2, reduction in crop combined with hang time lead with an increase in concentration of sugars in grape. This concentration of sugar plus the effects of climatic conditions over the vine will have an effect on the concentration of volatile compounds, described here as aromatic compound, present in grapes and therefore in final wine. This is the case of primary aromas detected in all the four cultivars. It has been reported that wines made from dehydrated grapes contain more terpenes and norisoprenoids than controls (Moreno et al. 2008), in the same way crop reduction increased free and potential volatile terpenes (Reynolds et al. 1989 and Reynolds et al. 2007).

Varietal aromas.

Terpenes like linalool, geraniol, terpineol and citronellol were present in some cultivar with some exceptions. In Pinot gris, only citronellol and terpinolene were found. The first was only present in the last hang time in 2011 but not detected in 2012, while the second had a decrease with increased hang time but only present in half crop in 2011, and in both crop levels at T2 in 2012. The effect of dehydration played a role in the development of this compound (Moreno et al. 2008). Citronellol in Cabernet Franc decreased with hang time until it reached zero at T2; this reduction in concentration could be explained due to possible changes in the metabolism in grape when desiccation occurs (Kays 1997; Wang and Brennan 1995). In Cabernet Sauvignon, Citronellol was only detected in 2012 and only in reduced crop in Cabernet franc 2011. Citronellol, as well as other terpenes, is a compound that normally have an odour impact (Mateo and Jiménez 2000), and even though their OAV values were slightly > 1 is possible that other compounds overshadowed them in this situation.

Linalool, geraniol and terpineol were detected in Riesling, the last two only present in 2011; Linalool increased in concentration with hang time and either between crop levels in 2011 but only present in samples of T2 in 2012 in higher concentration; this increase in concentration while increase hang time may be a reflection of dehydration process occurring in grape. For the particular case in 2012 the production of Linalool could be related to a desiccation occurred in the grapes at this stage possible to the action of *B. cinerea* (Sponholz 1993); in 2012 a reduction at T2 in berry weight and yield (Table 2.4.4) was a particular characteristic for this cultivar at this stage. Concentration for this compound is higher than reported in literature, 4.7-307 ppb for young white wines (Escudero et al. 2004; Guth 1997).

Comparing with the results of must analysis (Figure 3.1) for Riesling, the higher concentrations were found at T2 in comparison with the other hang times, this is a reflection for this compound in wine. However, linalool in must was present in T0 and T1; the action of commercial enzyme glucosidase over the samples released this aromatic aglycon. This is in agreement with the increase of terpene concentration and the use of pectolytic enzymes (Versini et al. 1981) Must samples were collected before raking of must, It was reported that glycosylated precursors of linalool, geraniol, and others were reduced when clarification was imposed in must (Moio et al. 2004), this could also explain why those levels of glycoside linalool were not present in wine.

Geraniol in Riesling was not detected in 2012, but in 2011 a decline in concentration was noted at each hang time, with values similar to the reported in literature at 0.221 mg/L (Francis and Newton 2008). Even though analysis of must detected the presence of this compound in 2012, this was not reflected in the wine. Enzymatic treatment in must was significantly higher in samples of must than in the non-enzymatic samples.

Terpineol as was geraniol, which was just detected in 2011 with an increase in concentration with increased hang time. Noble rot following an infection of *B. cinerea* generates the conversion of linalool, geraniol and nerol to less volatile compounds like terpineol (Bakker and Clarke 2012). This could explain this increase in 2011, a wetter year during harvest than 2012, dry and cold, which made less susceptible for infections.

The must analysis in 2012 found an increase in concentration of this component with respect of hang time with an increase in concentration in enzymatic treatment with respect to the non-enzymatic. An effect other than viticultural practices affected the presence of this terpene in final wine, possibly clarification after fermentation.

These terpenes in wine had OAV´s > 1, making them odour active where present.

Terpinolene, a component with an odour quality as plastic/pine aroma, detected in reduced crop samples at all hang time in Pinot gris 2011 and only in T2 in 2012 could be generated by a transformation due to an acid-catalyzed rearrangement of nerol and linalool to another terpene (Marais 1983). Nerol and linalool are more sensitive to acid conditions and temperature (Marais 1983), producing a less aromatic compound like terpinolene. In both years an increase in TA with increased time could have elicited the formation of this component.

In Riesling, this compound was mainly present during 2011, a warmer wet year, with an increase in the half crop treatments, and an increase in the concentration with time. In 2012 it was only detected at T2 at levels closer to those found in 2011. The TA at this stage was similar to 2011; this increase in TA could be linked with the formation of terpinolene.

Damascenone was detected in all cultivars in all treatments. A constant reduction occurred in all cultivars with increased hang time. It has been reported that carotenoid profiles, which are precursors for damascenone and other norisoprenoids, in grapes that were thermo-tunnel dried, declined in concentration under this treatment (Chkaiban et al. 2007). The generation of norisoprenoids from the breakdown of carotenoids can proceed *via* chemical, photochemical and oxidase-coupled degradation, the fact that the majority of norisoprenoids in grapes have 13 carbons suggests that it is regulated by enzymatic cleavage (Dunlevy et al. 2009). Possibly the effect of stress due to desiccation present in grapes hung for longer periods, particularly at T2, could be the responsible for a detriment in the capacity of enzymes to generate the breakdown product. In general, the OAV´s for damascenone were > 1, it does not mean that it made a big impact over the aroma profile in the final wine,

rather it is just considered as a component that add nuances to different cultivars (Winterhalter et al. 1990).

Secondary Metabolites

Esters

Among the group of esters found in the wines, the four most abundant were detected: ethyl acetate, isoamyl acetate, ethyl caproate and ethyl caprylate (Avakyants et al. 1981). The majority were impacted by increasing hang time than reducing crop. Whilst ethyl acetate and ethyl butyrate in almost all cases increased with increases in hang time, ethyl caproate and hexyl acetate were reduced. They are reported to be formed during yeast fermentation, although could be present in small amounts in grapes (Ralph and Madery 1986). Differences in sugar concentration prior fermentation particularly at T2 and subsequent fermentation lead with the generation of these secondary metabolites.

In general, effect of hang time was greater on this group of esters than was crop reduction. These increases may have been linked to the increase of sugar concentration by dehydration (Bellincontron et al. 2004; Constantini et al. 2006), and subsequent fermentation and production of ethanol and high alcohols. The last along with acetyl-coA, eliciting the synthesis of acetates of higher alcohols (Zamora 2009), but changes in esters could also be generated by a re-establishment of chemical equilibrium relative to the percentage of ethanol and acids in the wines immediately after fermentation (Bakker and Clerk 2012), coupled with changes in berry metabolism when water loss occurred (Zamboni et al. 2008)

Two different groups of esters were detected in wines, the acetate of higher alcohols like ethyl acetate, isoamyl acetate and hexyl acetate, and esters of fatty acids and ethanol like ethyl butyrate, caproate, caprylate, heptanoate, and decanoate.

Among the first group, synthetized from acetyl-coA and the different higher alcohol (Zamora 2009) the effect of increase in sugar concentration followed by production of ethanol and their respective high alcohol could be responsible for the changes in concentration for each component.

The increase of ethyl acetate (figure 3.7) in almost all wines is confirmed by the findings of Zamboni et al. (2008), and linked to a metabolic

stress response when 11.7% of water lost was present in grape. However, a decrease in Riesling was detected in 2012 relative to extended hang time, but just at T2, berry weight at this time was also lower in comparison with the other two hang times. T2 during 2011 in Riesling had the most elevated concentration of ethyl acetate, possibly giving a more intense pineapple odour.

Isoamyl acetate (figure 3.8), a compound related to banana odour quality, was impacted by both factors in different ways; in white cultivars, concentration was reduced with increased hang time which is consistent with the reduction of Isoamyl alcohol (figure 3.11) in the same cultivars, while in red cultivars, crop reduction led to increases in Cabernet franc, Isoamyl alcohol had not significant change suggesting a different effect, possibly and increase of acetates of carboxylic acids like acetic acid linked to an increase of volatile acidity, and reductions in Cabernet Sauvignon. Vintage had an impact in white cultivars with a marked increase in concentration in 2012, linked to the significant increase in Brix in grapes and alcohol in final wines; red cultivars remained unchanged.

Hexyl acetate (figure 3.9), with an herbal-fruity odour quality, was only present in white cultivars, in agreement with the literature (Baumes et al. 1986; Rapp and Mandery 1986) and was reduced in concentration with increased hang time. Concentrations were in the range of 5.4 – 7.3 mg/L reported by Rapp and Mandery (1986). No vintage effect was apparent for this component.

The second group of esters of fatty acids and ethanol are synthesised from the different acyl-CoA and ethanol (Zamora 2009). Effects over the enzymatic pathway through the formation of these esters in vinification and the production of ethanol could explain the differences found in this experiment. During the vinification process, the grape aliphatic compounds are depleted and converted to alcohols and esters, usually with positive sensory attributes (Palomo et al. 2007). The lipoxygenase pathway involves a series of enzymes that oxidize and cleave polyunsaturated fatty acids to yield aldehydes, which are reduced subsequently to alcohols and esterified in the presence of short chain carboxylic acids such as acetic acid (Dunlevy et al. 2009). The different enzyme actions in this pathway are initiated when grapes

are crushed and enzymes are in contact with fatty acids in the presence of oxygen (Chkaiban et al. 2007).

Ethyl butyrate, a compound related to an apple odour quality was always found with OAV´s < 1, which suggests it was not odour active.

Ethyl caproate was found in higher concentrations in white cultivars than red ones, which matches the range described by Bakker and Clarke (2012); i.e. 0.06 to 2 mg/L in whites and 0.06 to 0.13 mg/L in reds. Two aspects marked this compound; while in almost all wines it was reduced with increases in hang time this could be linked to a reduction of fatty acids present in grape previous fermentation particularly at the last stage of hang, the effect of vintage was also evident. Higher concentrations in 2012 were detected in Pinot gris and Riesling in comparison with 2011, and general reductions were observed in their concentrations in 2012 with respect of 2011 for Cabernet franc and Cabernet Sauvignon. The reduction in red wines could be linked also to a hydrolysis of this ester due to malolactic fermentation or the action of lactobacillus bacteria (Costantini et al. 2009), the pH of these wines were higher than 3.4 (tables 2.4.9 – 2.4.12), generating a feasible environment for their growing (Davis et al. 1986), pH was increased with respect of time while concentration reduced.

Ethyl caprylate (figure 3.10) was found in higher concentrations in white wines with respect to the values obtained in literature (1.10 to 5.10 mg/L; Bakker and Clerk 2012) but lower in reds (1 to 6 mg/L; Bakker and Clerk 2012), and this suggested a higher odour activity for white cultivars, particularly Riesling, since Pinot gris lacked its presence in 2011. Cabernet franc had increased concentration for this compound with increases in hang time, but Cabernet Sauvignon was not impacted; again vintage made the difference with higher concentrations in 2011 in the red cultivars than 2012.

Ethyl heptanoate, a compound with a fruity odour quality, was mostly detected in red cultivars with an increase in concentration with increased hang time; however, for this component all OAV´s were < 1, suggesting it was not odour active.

The same behaviour was observed for diethyl succinate; even though was more impacted in red cultivars than whites, in both cases it had an OAV >

1. Ethyl decanoate was not odour active for red cultivars, but was slightly > 1 for whites, with just a slight increase with extended hang time during 2012.

Higher alcohols

The production of higher alcohols is linked to the production of ethanol in wines; however, they do not follow a particular trend as esters can. They can be derived from amino acids (Nykänen 1986). Some enzymes are responsible for the degradation of aldehydes to alcohols like alcohol dehydrogenases (ADH), which catalyse the reversible reduction of aliphatic aldehydes to alcohols (Dunlevy et al. 2009). It has been reported that in grapes, stress conditions have been associated with an increase in ADH activity and *ADH* gene expression (Dunlevy et al. 2009). Therefore ADH in grape berries can be activated at certain levels of water loss (Costantini et al. 2006).

This could explain the higher concentrations found in some compounds like isobutyl alcohol; this component was reduced in Pinot gris in 2011 with increased hang time, which is beneficial, since it potentially would reduce the pungency and bitterness to a more pleasant level (< 300 mg/L; Bakker and Clerk 2012).

Isobutyl alcohol was not present in Pinot gris in 2012. Higher concentrations were found in Riesling in 2011 in comparison with literature (6-174 mg/L; Bakker and Clerk 2012), but was ≈ 300 mg/L in 2012; in both years it was only present in T2 wines, which could be related to the presence of sour rot, which elicits formation of this compound in Riesling grapes (Zoecklein et al. 2010). Similar concentrations for this alcohol were found in red cultivars in both years, without any trend with respect to viticultural treatments; just Cabernet Sauvignon lacked of this in 2011.

Phenyl ethyl alcohol (figure 3.12) was observed in all wines and did not have a general trend between cultivars; it was found at levels reported in literature (9 – 153 mg/L in reds and 13.9 – 86.8 in whites; Escudero et al. 2004; Lopez et al. 2003; Murat et al. 2001). OAV´s for white cultivars were slightly > 1 but higher for red cultivars, which suggests that it had a greater impact in red cultivars in terms of its honey, spice and rose-lilac odour.

Isoamyl alcohol (figure 3.11), with a malt-burn whiskey odour quality, was present in red cultivars in 2011 with concentrations higher with respect to

literature and OAV´s > 40. Generation of this compound may have occurred due to spoilage during extended hang time; Cabernet franc, however, benefitted by increased hang time with a reduction in this alcohol.

Hexanol, a higher alcohol with a leaf-grassy odour quality, was present all cultivars; in whites their concentrations were just high enough to make the compound odour active; but in reds the concentrations were higher than reported in literature, 2.1 – 13.2 mg/L, likely generating an increase in the leaf-grassy aroma (Guth 1997). In these reds decreases in hexanol concentration during increased harvest date was likely beneficial to the flavour of wines.

Other higher alcohols were found sporadically in some wines and their presence could be related to on-vine spoilage of grapes during extended hang time. *B cinerea* could have enhanced the production of polyols during berry shrinking and loss of water (Sponholz 1993). Production of high amounts of nonanol in Cabernet Sauvignon wines occurred, and increased with hang time in 2011, but was only detected in 2012 in the half crop/T2 samples. This is related to the climatic conditions during harvest, 2011 wet and warm, while 2012 dry could explain the impact on spoilage to grapes and the formation of this alcohol.

Heptanol was also present in a few T2 samples in 2012 Riesling; this compound was also detected in samples of Cabernet franc 2012, but in both with OAV´s < 1. Similar trends were observed for Pinot gris with 1-octanol in 2012, with OAV´s < 1 making them non-odour active.

Volatile acids

The trend with volatile acids was the same in all cultivars, with a reduction in concentration with increased hang time; this reduction was likely beneficial since all compound from this group detected have an odour quality between sweat, cheese or even rancid fat. Hexanoic and decanoic acids had OAVs < 1, which suggests they were not odour active. It was reported that crop reduction increases concentration of volatile acids (Bravdo 1984); however, in this experiment, they were reduced primarily by extended hang time, which could be linked to a possible TA decrease and change in the acidic composition in grape consistent with Freeman and Kliewer (1983)

Other compounds.

Benzaldehyde is a compound associated with defects in wines (Bakker and Clerk 2012). This compound is probably formed by the oxidation of the benzyl alcohol or by action of the microorganisms on the aromatic amino acids (phenylalanine) or on the phenol compounds of the grape or on some secondary compounds such as phenyl acetic acid and p-hydroxybenzoic acid (Genovese et al. 2007). Benzaldehyde was just detected at T2 in a few wines like full crop Pinot gris in 2012 with an OAV of 10, suggesting this wine may have been faulty by the presence of microorganism such as *B. cinerea* in the grapes at the last hang time, while all other wines had OAV values $\leq$ 1. Diethyl acetal, a compound with a fruity-creamy odour quality, followed a similar trend to benzaldehyde with a high presence in T2 and with OAVs sufficiently high that it likely made a high impact on the wine sensory profile.

3.6 Conclusions

Viticultural treatments imposed by this study had impacts on the aroma composition of the wines produced. The increases in hang time led to a greater increase in Brix than crop reduction combined with commercial harvest (T0) as was explained in the previous chapter. This increase, coupled with the effect of delayed of harvest made a greater impact in aromatic compounds than crop reduction, possibly due to the high availability of sugars modifying chemical processes and transport phenomena in grape. In general, varietal aromas like terpenes in white cultivars were benefitted by an increase in concentration making them more varietal-like and intense. For the norisoprenoid damascenone, a particular decrease was linked with the increase of hang time. The effect of vintage impacted primary aroma compounds, with larger concentrations in some cases in 2012 than 2011, perhaps linked to the higher Brix values also detected in 2012 in all cultivars. Riesling was particularly less benefitted by vintage since a higher presence for these compounds were detected in 2011 than 2012.

Esters that developed after fermentation had unique behaviours but were always linked to the increase of hang time more so than crop reduction. In some cases, concentrations declined such as ethyl caproate, isoamyl acetate and hexyl acetate, while in other cases there were increases, e.g. ethyl caprylate. In some cases the effect of vintage was evident, with an

increase in concentration in 2012 in isoamyl acetate and ethyl caproate in white cultivars, but higher concentrations in 2011 for ethyl caprylate.

Volatile acids quantified in this experiment were reduced with the increase of hang time. This could be beneficial since all have aromas related to sweat, cheese and rancid. This reduction is concomitant with the reduction in TA in musts and wines.

Higher alcohols sometimes were impacted by delay of harvest. Even though ethanol concentrations were higher in all wines from extended hang times, this increase was not reflected in higher alcohols, and reductions were observed in phenyl ethyl alcohol or hexanol. For hexanol, the reduction was likely beneficial in red wines since a decrease in concentration would reduce the grassy-green odour which it characterizes.

The presence of some other compounds suggests minor spoilage by microorganisms, particularly at the T2 stage. Higher concentrations of isobutyl alcohol in Riesling could be linked to the presence of sour rot in grapes and increases in concentration of nonanol in Cabernet Sauvignon with increased hang time and particularly at T2 might suggest the presence of *B. cinerea*.

With all the information obtained during this experiment, it can be concluded that in general, extension in harvest had a better impact in general aroma profile in wine than crop reduction. This is linked not only with higher concentration of sugars, but also changes of metabolite components that could be either increase or reduce with the extension.

As in the case of chapter 2, the climatic conditions and vintage play an important roll on the development and presence of volatile components, which are important characteristics to take in account when this viticultural treatment is chosen. Faster decisions in vineyard plus selection of the most resistant cultivars will also be necessary to ensure the best aroma quality in wine.

3.7 Literature cited

Ahmed, E. M., Dennison, R. A., Dougherty, R. H., and Shaw, P. E. (1978). Flavor and odor thresholds in water of selected orange juice components. J. Agric. Food. Chem. 26:187-191.

Avakyants, S., Rastyannikov, E., Chernyaga, B., and Navrotskii, V. (1981). Khromato-mass-spektrometricheskoe issledovanie letuchikh vesnchestv vina. Vinodel. Vinograd. SSSR. 41:50-53.

Bakker, J., and Clarke, R. J. (2012). Wine flavour chemistry (2nd ed.). Chichester, West Sussex ; Ames, Iowa: Wiley-Blackwell.

Barata, A., González, S., Malfeito‐Ferreira, M., Querol, A., and Loureiro, V. (2008). Sour rot damaged grapes are sources of wine spoilage yeasts. FEMS Yeast Res. 8:1008-1017.

Baumes, R., Cordonnier, R., Nitz, S., and Drawert, F. (1986). Identification and determination of volatile constituents in wines from different vine cultivars. J. Sci. F. Agric. 37:927-943.

Bell, A., Ough, C., and Kliewer, W. (1979). Effects on must and wine composition, rates of fermentation, and wine quality of nitrogen fertilization of *Vitis vinifera* var. Thompson Seedless grapevines. Am. J. Enol. Vitic. 30:124-129.

Bellincontro, A., De Santis, D., Botondi, R., Villa, I., and Mencarelli, F. (2004). Different postharvest dehydration rates affect quality characteristics and volatile compounds of Malvasia, Trebbiano and Sangiovese grapes for wine production. J. Sci. Food Agr. 84:1791-1800.

Bonino, M., Schellino, R., Rizzi, C., Aigotti, R., Delfini, C., and Baiocchi, C. (2003). Aroma compounds of an Italian wine "Ruché" by HS–SPME analysis coupled with GC–ITMS. Food Chem. 80:125-133.

Borneman, A. R., Schmidt, S. A., and Pretorius, I. S. (2012). At the cutting-edge of grape and wine biotechnology. Trends in Genetics. 1:1006-1010

Boselli, E., Boulton, R. B., Thorngate, J. H., and Frega, N. G. (2004). Chemical and sensory characterization of DOC red wines from Marche (Italy) related to vintage and grape cultivars. J. Agr. Food Chem. 52:3843-3854.

Boulton, R. (1980). The relationship between total acidity, titratable acidity and pH in grape tissue. Vitis 19:113-120.

Bowen, A. J., & Reynolds, A. G. (2012). Odor potency of aroma compounds in Riesling and Vidal blanc table wines and icewines by gas Chromatography–Olfactometry–Mass spectrometry. J. Agri. F. Chem. 60:2874-2883.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., and Tabacman, H. (1984). Effect of crop level on growth, yield and wine quality of a high yielding Carignane vineyard. Am. J. Enol. Vitic. 35:247-252.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., and Tabacman, H. (1985). Effect of crop level and crop load on growth, yield, must and wine composition, and quality of Cabernet sauvignon. Am. J. Enol. Vitic. 36:125-131.

Burdock, G. A. (2010). Fenaroli's handbook of flavor ingredients CRC press, Sydney Australia.

Bureau, S. M., Razungles, A. J., and Baumes, R. L. (2000). The aroma of Muscat of Frontignan grapes: Effect of the light environment of vine or bunch on volatiles and glycoconjugates. J. Sci. F. Agri. 80:2012-2020

Chkaiban, L., Botondi, R., Bellincontro, A., Santis, D., Kefalas, P., and Mencarelli, F. (2007). Influence of postharvest water stress on lipoxygenase and alcohol dehydrogenase activities, and on the composition of some volatile compounds of Gewürztraminer grapes dehydrated under controlled and uncontrolled thermohygrometric conditions. Austral. J. Grape and Wine. Res. 13:142-149.

Comuzzo, P., Tat, L. and Battistutta, F. (2006). Yeast derivatives (extracts and autolysates) in winemaking: Release of volatile compounds and effects on wine aroma volatility. Food Chem. 99:217-230.

Cordner, C., and Ough, C. (1978). Prediction of panel preference for Zinfandel wine from analytical data: Using difference in crop level to affect must, wine, and headspace composition. Am. J. Enol. Vitic. 29:254-257.

Constantini, V., Bellincontro, A., De Santis, D., Botondi, R., and Mencarelli, F. (2006). Metabolic changes of Malvasia grapes for wine production during postharvest drying. J. Agr. Food Chem. 54:3334-3340.

Costantini, A., García-Moruno, E., and Moreno-Arribas, M. V. (2009). Biochemical transformations produced by malolactic fermentation. *In* Wine chemistry and biochemistry (ed) Moreno-Arribas M.V. pp. 27-57 Springer, New York

Cullère, L., Escudero, A., Cacho, J., and Ferreira, V. (2004) Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. J. Agric. Food. Chem. 52:1653-1660

Davis, C., Wibowo, D., Lee, T., and Fleet, G. (1986). Growth and metabolism of lactic acid bacteria during and after malolactic fermentation of wines at different pH. App. Enviro. Microb. 51:539-545.

Dimitriadis, E., and Williams, P. (1984). The development and use of a rapid analytical technique for estimation of free and potentially volatile monoterpene flavorants of grapes. Am. J. Enol. Vitic. 35:66-71.

Dunlevy, J., Kalua, C., Keyzers, R., and Boss, P. (2009). The production of flavour and aroma compounds in grape berries. Grapevine Mol. Phy. Biot. 293-340.

Edson, C.G., Howell, G.S., and Flore, J. (1993). Influence of crop load on photosynthesis and dry matter partitioning of Seyval grapevines I. single leaf and whole vine response pre-and post-harvest. Am. J. Enol. Vitic. 44:139-147.

Escudero, A., Gogorza, B., Melus, M., Ortin, N., Cacho, J., and Ferreira, V. (2004). Characterization of the aroma of a wine from Maccabeo. key role played by compounds with low odor activity values. J. Agric. F. Chem. 52:3516-3524.

Etievant, P. (1991). Wine. In Volatile Compounds in Foods and Beverages. Maarse Henk (ed), pp 483-546, Food Science and Technology. New York.

Fan, W., Tsai, I., and Qian, M.C. (2007). Analysis of 2-aminoacetophenone by direct-immersion solid-phase microextraction and gas chromatography–mass spectrometry and its sensory impact in Chardonnay and Pinot gris wines. F. Chem. 105:1144-1150.

Fang, F., Li, J., Zhang, P., Tang, K., Wang, W., Pan, Q., and Huang, W. (2008). Effects of grape variety, harvest date, fermentation vessel and wine ageing on flavonoid concentration in red wines. Food Res. Int. 41:53-60.

Ferreira, V., López, R., and Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. J. Sci. Food Agri. 80:1659-1667.

Fleet, G.H. (1993). Wine: Microbiology and Biotechnology CRC Press. Sydney Australia.

Francis, I., and Newton, J. (2008). Determining wine aroma from compositional data. Austral. J. Grape and Wine Res. 11:114-126.

Freeman, B.M. and Kliewer, M. (1983). Effect of irrigation, crop level and potassium fertilization on Carignane vines. II. grape and wine quality. Am. J. Enol. Vitic*. 34*:197-207.

Genovese, A., Gambuti, A., Piombino, P., and Moio, L. (2007). Sensory properties and aroma compounds of sweet Fiano wine. *Food Chem. 103*:1228-1236.

Guerzoni, E., and Marchetti, R. (1987). Analysis of yeast flora associated with grape Sour rot and of the chemical disease markers. Appl. Env. Microbiol. 53:571-576.

Guth, H. (1997). Identification of character impact odorants of different white wine varieties. J. Agri. Food Chem. 45:3022-3026.

Hardy, P. (1970). Changes in volatiles of muscat grapes during ripening. Phytochemistry 9:709-715.

Herbert, P., Cabrita, M. J., Ratola, N., Laureano, O., and Alves, A. (2005). Free amino acids and biogenic amines in wines and musts from the Alentejo region. evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage. J. Food Eng. 66:315-322.

Himelrick, D.G. (2003). Handling, storage and postharvest physiology of Muscadine grapes: A review. Small Fruits Review 2:45-62.

Howard, K.L., Mike, J.H., and Riesen, R. (2005). Validation of a solid-phase microextraction method for headspace analysis of wine aroma components. Am. J. Enol. Vitic. 56:37-45.

Jackson, D., and Lombard, P. (1993). Environmental and management practices affecting grape composition and wine quality-a review. Am. J. Enol. Vitic. 44:409-430.

Jackson, D., and Schuster, D. (2001). The production of grapes & wine in cool climates. Aotearoa, New Zealand: Daphne Brasell Associates Ltd and Gypsum Press.

Kays, S.J. (1997). Stress in harvested products, in Postharvest Physiology in Perishable Plant Products, Ed by Kays SJ. Exon Press. Athens, GA, pp 335–408.

Keith, E. S., and Powers, J. J. (1968). Determination of flavor threshold levels and sub‐threshold, additive, and concentration effects. J. F. Sci. 33:213-218

Kliewer, W.M., Howarth, L., and Omori, M. (1967). Concentrations of tartaric acid and malic acids and their salts in *Vitis vinifera* grapes. Am. J. Enol. Vitic. 18:42-54.

Kliewer, W.M. (1971). Effect of day temperature and light intensity on concentration of malic and tartaric acids in *Vitis vinifera* L. grapes. J. Am. Soc. Hort. Sci. 96:372-377.

Kliewer, W.M., and Ough, C. (1970). The effect of leaf area and crop level on the concentration of amino acids and total nitrogen in Thompson Seedless grapes. Vitis 9:196-206.

Lea, P., Naes, T., and Rødbotten, M. (1997). Analysis of variance for sensory data. Wiley and Sons. West Sussex, England.

López, R., Ortín, N., Pérez-Trujillo, J. P., Cacho, J., and Ferreira, V. (2003). Impact odorants of different young white wines from the Canary Islands. J. Agric. F. Chem. 51:3419-3425.

Marais, J. (1983). Terpenes in the aroma of grapes and wines: A review. S. Afr. J. Enol. Vitic. 4:49-60.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., and Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. J. Agr. Food Chem. 10:4009-4017.

Moio, L., Ugliano, M., Gambuti, A., Genovese, A., and Piombino, P. (2004). Influence of clarification treatment on concentrations of selected free varietal aroma compounds and glycoconjugates in Falanghina (*Vitis vinifera* L.) must and wine. Am. J. Enol. Vitic. 55:7-12.

Moreno, J. J., Cerpa-Calderón, F., Cohen, S. D., Fang, Y., Qian, M., and Kennedy, J. A. (2008). Effect of postharvest dehydration on the composition of Pinot noir grapes "*Vitis vinifera*" L. and wine. Food Chem. 109:755-762.

Morris, J.R., and Cawthon, D. (1982). Effect of irrigation, fruit load, and potassium fertilization on yield, quality, and petiole analysis of concord (*Vitis labrusca* L.) grapes. Am. J. Enol. Vitic. 33:145-148.

Murat, M., Tominaga, T., and Dubourdieu, D. (2001). Impact of some components on Bordeaux roses and Clairets aroma. Int. J. Vine Wine Sci. 35:99-105

Nelson, R.R., and Acree, T.E. (1978). Concord wine composition as affected by maturity and processing technique. Am. J. Enol. Vitic. 29:83-86.

Nykänen, L. (1986). Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. Am. J. Enol. Vitic. *37*:84-96.

Ortega-Heras, M. González-SanJose, M. L. and Beltran, S. (2002). Aroma composition of wine studied by different extraction methods. Anal. Chim. Act*, 458*:85-93.

Pineau, B., Barbe, J., van Leeuwen, C., and Dubourdieu, D. (2007). Which impact for β-damascenone on red wines aroma? J. Agr. F. Chem. 55:4103-4108.

Rapp, A., and Mandery, H. (1986). Wine aroma. *Experientia, 42*:873-884.

Rapp, A. (1998). Volatile flavor of wine: Correlation between instrumental analysis and sensory perception. Food 42:351-363.

Reynolds, A.G., Price, S.F., Wardle, D.A. and Watson, B.T (1994). Fruit environment and crop level effects on Pinot noir. I. vine performance and fruit composition in British Columbia. Am. J. Enol. Vitic. 45:452-459.

Reynolds, A.G., Yerle, S., Watson, B.T., Price, S.F., and Wardle, D.A. (1996). Fruit environment and crop level effects on Pinot noir. III. composition and descriptive analysis of Oregon and British Columbia wines. Am. J. Enol. Vitic. 47:329-339.

Reynolds, A.G., and Wardle, D.A. (1989). Impact of various canopy manipulation techniques on growth, yield, fruit composition, and wine quality of Gewürztraminer. Am. J. Enol. Vitic. 40:121-129.

Reynolds, A. G., Schlosser, J., Sorokowsky, D., Roberts, R., Willwerth, J., and de Savigny, C. (2007). Magnitude of viticultural and enological effects. II. relative impacts of cluster thinning and yeast strain on composition and sensory attributes of Chardonnay musqué. Am. J. Enol. Vitic*. 58*:25-41.

Ruth, J. H. (1986). Odor thresholds and irritation levels of several chemical substances: A review. Am. Ind. Hyg. Ass. J. 47:142-151

Somers, T. (1975). In search of quality for red wines. Food Tech. Austral. 27:49-56.

Sponholz, W. (1993). Wine spoilage by microorganisms. *In* Wine Microbiology and Biotechnology, Fleet (Ed), pp. 395-420. Harwood Academic Publisher, Chur, Switzerland.

Tominaga, T., Baltenweck-Guyot, R., Peyrot des Gachons, C., and Dubourdieu, D. (2000). Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. Am. J. Enol. Vitic. 51:178-181.

Versini, G., Inama, S., and Sartori, G. (1981). A capillary column gas chromatographic research into the terpene constituents of ‘‘Riesling renano’’(Rhine Riesling) wine from Trentino Alto Adige: Their distribution within berries, their passage into must and their presence in the wine according to different wine-making procedures. organoleptic considerations. Organoleptic Considerations.Vini. Ital. 23:189-211.

Wang, N., and Brennan, J. (1995). Changes in structure, density and porosity of potato during dehydration. J. Food Eng. 24:61-76

Winkler, A.J., and Williams, W. (1940). The heat required to bring Tokay grapes to maturity. Proc. Am. Soc. Hortic. Sci. 37:650-652.

Winterhalter, P., Sefton, M., and Williams, P. (1990). Volatile C13-norisoprenoid compounds in Riesling wine are generated from multiple precursors. Am. J. Enol. Vitic. 41:277-283.

Zalacain, A., Marín, J., Alonso, G., and Salinas, M. (2007). Analysis of wine primary aroma compounds by stir bar sorptive extraction. Talanta 71:1610-1615.

Zamboni, A., Minoia, L., Ferrarini, A., Tornielli, G. B., Zago, E., Delledonne, M., and Pezzotti, M. (2008). Molecular analysis of post-harvest withering in grape by AFLP transcriptional profiling. J. Exp. Bot. 59:4145-4159.

Zamora, F. (2009). Biochemistry of alcoholic fermentation. *In* Wine chemistry and biochemistry (ed) Moreno-Arribas M.V. pp. 3 - 26 Springer, New York

Zhang, M., Xu, Q., Duan, C., Qu, W., and Wu, Y. (2007). Comparative study of aromatic compounds in young red wines from Cabernet Sauvignon, Cabernet franc, and Cabernet Gernischet varieties in China. J. Food Sci. 72:C248-C252.

Zoecklein, B., Williams, J., and Duncan, S. (2010). Effect of sour rot on the composition of White Riesling (Vitis vinifera L.) grapes. Small Fruit Rev. 1:63-77.

Appendix Chapter 3

Table 3.4.1 Impact of hang time and crop level treatments on the wine volatile compounds [b] of Pinot Gris 2011-2012

Year	Factor	Ethyl butyrate	1-Hexanol	Isobutyl alcohol	Hexyl acetate	Ethyl caprylate	Phenyl ethyl alcohol	Octanoic acid	Diethyl acetal	Citronellol	Isoamyl alcohol	Ethyl caproate
2011	**Crop Level**											
	Full	0.17	56.9	1234.4	3.9	----	22.6	15.9	312.5	0.067	1431	6.6
	Half	0.14	55.3	243.4	3.6	----	16.7	15.4	313.2	0.066	1411	7.8
	Significance[a]	ns	ns	****	ns	----	ns	ns	ns	ns	ns	ns
	Hang time											
	T0	0.18	58.1	1127.5 a	5.8 a	----	24.7	17.2 a	0 b	0 b	1444	7.2 ab
	T1	0.15	55.4	742.3 b	5.5 a	----	16.5	18.9 a	0 b	0 b	1430	10.3 a
	T2	0.14	55.0	346.8 c	0 b	----	17.7	10.7 b	938.6 a	0.199 a	1390	4.1 b
	Significance[a]	ns	ns	****	****	----	ns	**	****	****	ns	*
	Interaction	ns	ns	****	ns	----	ns	ns	ns	ns	ns	ns
2012	**Crop Level**											
	Full	0.31	58.1	----	3.9	25.1	22.4	84.4	318.3	0	3807	36.1
	Half	0.19	0.00	----	2	25.2	14.0	25.8	313.6	0	1550	9.6
	Significance[a]	ns	****	----	****	ns	ns	ns	ns	----	ns	ns
	Hang time											
	T0	0.2 b	27.5 b	----	6.1 a	28.5	15.8	37.4	0 b	0	1520 b	12.9
	T1	0.18 b	27.6 b	----	2.9 b	24.0	16.2	28.9	0 b	0	1635 b	10.8
	T2	0.37 a	32.1 a	----	0 c	22.8	25.5	98.9	948 a	0	4880 a	44.9
	Significance[a]	ns	****	----	****	ns	ns	ns	****	----	ns	ns
	Interaction	*	****	----	****	*	ns	ns	ns	----	ns	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test. [b] concentrations in mg/L

Table 3.4.1 (continuation)

Year	Factor	Hexano-ic acid	Ethyl phenyl acetate	Ethyl decanoate	1-Octanol	2-Phenethyl acetate	Diethyl succinate	Isoamyl acetate	Terpino-lene	Ethyl acetate	Benz-aldehyde	Damas-cenone	Decanoic acid
2011	**Crop Level**												
	Full	24.1	0.337	0.463	----	11.3	0 y	7.5	0 y	207.9	-	0.316	25.4
	Half	11.8	0.334	0.189	----	10.9	3.7 z	7.4	0.298 z	149.1	-	0.07	25
	Significance[a]	ns	ns	ns	----	ns	****	ns	****	ns	-	ns	ns
	Hang time												
	T0	30.8	0 b	0.599	----	11.4	2.9 a	7.9 a	0.156 a	211.8	-	0.358	27.2 a
	T1	12.7	0.506 a	0.270	----	11.2	2.6 b	8.7 a	0.150 b	177.3	-	0.131	36.2 a
	T2	10.4	0.501 a	0.110	----	10.7	0 c	5.8 b	0.141 c	146.3	-	0.09	12.1 b
	Significance[a]	ns	****	ns	----	ns	****	**	****	ns	-	ns	**
	Interaction	ns	ns	ns	----	ns	****	ns	****	ns	-	ns	ns
2012	**Crop Level**												
	Full	48.6	0.288	0.647	0.229	11.9	2.9	19.2	0.120 y	172.7	5.5	0.247	48.5
	Half	17.7	0	0.236	0.213	10.9	1.1	10.7	0.089 z	195.2	0.9	0.534	43.1
	Significance[a]	ns	***	ns	ns	ns	ns	ns	*	ns	ns	***	ns
	Hang time												
	T0	19.8	0 b	0.217	0.132	11.2	0 b	15.9	0 b	183	0 b	0.431	64.9 a
	T1	16	0 b	0.196	0.204	10.9	0 b	9.9	0 b	171.1	0 b	0.437	54.4 b
	T2	63.7	0.432 a	0.912	0.327	12.2	6 a	19	0.314 a	197.7	9.6 a	0.303	18.2 c
	Significance[a]	ns	***	ns	ns	ns	*	ns	****	ns	*	ns	****
	Interaction	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	*

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test. [b] concentrations in mg/L

Table 3.4.2. Impact of hang time and crop level treatments on the wine volatile compounds [b] of Riesling 2011-2012

Year	Factor	Ethyl butyrate	1-Hexanol	Isobutyl alcohol	Hexyl acetate	Ethyl caprylate	Phenyl ethyl alcohol	Octanoic acid	Diethyl acetal	1-Heptanol	Linalool	Citronel-lol	Geraniol	Isoamyl alcohol
2011	**Crop Level**													
	Full	0.15	55.2	268	3.6	15.9	17.3	17.1	314.8	----	0.57	0.164	0.023	1320.0
	Half	0.15	37.0	237	5.5	16.5	16.9	19.6	0	----	0.62	0.161	0.083	1344.5
	Significance[a]	ns	****	*	****	ns	ns	ns	****	----	ns	ns	****	ns
	Hang time													
	T0	0.15	55.8 a	0 b	5.7 a	24.1 a	16.7	26 a	0 b	----	0.48 b	0 b	0.083 a	1316.6
	T1	0.14	55.2 b	0 b	5.3 b	6.9 b	16.7	11 c	0 b	----	0.61 ab	0.215 a	0.041 b	1233.4
	T2	0.15	27.3 c	757.6 a	2.6 c	17.6 a	17.9	18 b	472.3 a	----	0.7 a	0.273 a	0.035 b	1446.8
	Significance[a]	ns	****	****	****	***	ns	****	****	----	****	***	****	ns
	Interaction	ns	****	*	****	**	ns	*	****	----	ns	ns	****	ns
2012	**Crop Level**													
	Full	0.16	55.3	234	5.7	12.8	15.9	29.3	----	0.57	0.37	0.054	0	1286.2
	Half	0.05	18.2	0	3.8	11.3	15.5	27.4	----	0	0.3	0	0	1268.2
	Significance[a]	****	****	****	****	ns	**	ns	----	****	ns	****		ns
	Hang time													
	T0	0.08 b	27.8 b	0 b	5.7 a	13.7 a	14.9 b	34.7 a	----	0 b	0 a	0 b	0	1146.9 b
	T1	0.09 b	27.6 b	0 b	5.6 a	12.3 ab	16 a	30.4 b	----	0 b	0 a	0 b	0	1413.7 a
	T2	0.16 a	54.9 a	351.4 a	2.9 b	10.1 b	16.2 a	19.8 c	----	0.86 a	1 b	0.082 a	0	1271.1ab
	Significance[a]	****	****	****	****	ns	****	****	---	****	****	****	---	ns
	Interaction	****	****	****	****	ns	*	**	---	****	ns	****	---	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test. [b] concentrations in mg/L

Table 3.4.2. (Continuation)

Year	**Factor**	Ethyl caproate	Nonyl aldehyde	Hexano-ic acid	Ethyl phenyl acetate	Ethyl decan-oate	Terpin-eol	2-Phen-ethyl acetate	Isoamyl acetate	Terpino-lene	Ethyl acetate	Ethyl heptan-oate	Benzal-dehyde	Damas-cenone	Deca-noic acid
2011	**Crop Level**														
	Full	4.6	0.144	11.3	0.51	0.28	1.03	10.7	6.5	0.109	169.4	0	0	0.102	29.3
	Half	4.7	0	12.9	0.49	0.178	0.94	10.8	7.1	0.309	162.8	0	0	0.092	28.4
	Significance[a]	ns	****	ns	*	ns	ns	ns	ns	****	ns	---	---	ns	ns
	Hang time														
	T0	6.6 a	0 b	14.9 a	0.5	0.223 ab	0.95	10.8	7.7 a	0.153 b	145.4 b	0	0	0.125 a	33.8 a
	T1	1.8 b	0.217 a	9.9 b	0.493	0.072 b	0.91	10.7	5.7 b	0.158 b	138.7 b	0	0	0.064 b	12.9 b
	T2	5.4 a	0 b	11.6 b	0.506	0.394 a	1.1	10.8	7 a	0.316 a	214.3 a	0	0	0.101 ab	39.7 a
	Significance[a]	***	****	**	ns	*	ns	ns	**	****	*	---	---	*	****
	Interaction	*	****	ns	ns	ns	ns	ns	ns	****	ns	---	---	ns	**
2012	**Crop Level**														
	Full	8.1	0	15.9	0	0.092	0	11	14.4	0.115	139.2	0.088	0.449	0.545	40.3
	Half	7.5	0	14.7	0.16	0.071	0	11	11.5	0.104	145.3	0.077	0.140	0.335	42.5
	Significance[a]	ns	---	ns	****	ns	---	ns	***	****	ns	ns	***	**	ns
	Hang time														
	T0	8.8	0	18.9 a	0 b	0.094	0	10.8 b	11.0 b	0 b	155.1 a	0 b	0 b	0.653 a	56.7 a
	T1	8.1	0	15.3 b	0 b	0.085	0	11.1 a	11.4 b	0 b	150.2 a	0 b	0 b	0.416 b	49.6 a
	T2	6.6	0	11.7 c	0.242 a	0.066	0	11.1 a	16.5 a	0.329 a	121.5 b	0.248 a	0.884 a	0.25 c	17.9 b
	Significance[a]	ns	---	***	****	ns	---	****	****	****	****	****	****	***	***
	Interaction	ns	---	ns	****	ns	---	ns	****	****	*	ns	***	*	**

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test. [b] concentrations in mg/L

Table 3.4.3 Impact of hang time and crop level treatments on the wine volatile compounds [b] of Cabernet Franc 2011-2012

Year	**Factor**	Ethyl butyrate	1-Hexanol	Isobutyl alcohol	Ethyl caprylate	Phenyl ethyl alcohol	Octanoic acid	Diethyl acetal	1-Heptanol	Decanal	Citronellol
2011	**Crop Level**										
	Full	0.05 y	37.4	633.0	2.8	20.1	5.5	314.1	----	----	0
	Half	0.14	37.4	740.4	2.7	21.0	5.7	314.5	---	---	0.145
	Significance[a]	****	ns	****	ns	ns	ns	ns	---	---	****
	Hang time										
	T0	0.07 b	56.4 a	683.3 b	1.9 b	21.7 a	5.9 a	0 b	---	---	0.102 a
	T1	0.07 b	55.8 b	743.7 a	2.9 ab	21.7 a	5.8 a	0 b	---	---	0.115 a
	T2	0.14 a	0 c	633 c	3.4 a	18.2 b	5 b	942.9 a	----	----	0 b
	Significance[a]	****	****	****	ns	**	**	****	---	---	***
	Interaction	****	ns	****	ns	ns	ns	ns	---	---	***
2012	**Crop Level**										
	Full	0.13	41.4	713.0	0.52	13.1	5.6	233.5	0.41	0.112	0.067
	Half	0.13	41.5	698.8	0.74	13.3	5.9	233.3	1.04	0	0.190
	Significance[a]	ns	**	ns	*	ns	ns	ns	****	****	*
	Hang time										
	T0	0.13	55.7 a	712.8	0.5 b	13.2	5.8	0 b	0.84 b	0 b	0.092 b
	T1	0.13	54.9 b	708.5	0.53 b	13.3	6	0 b	0 c	0.149 a	0.250 a
	T2	0.13	0 c	691.7	0.98 a	13.1	5.2	933.5 a	1.64 a	0 b	0 b
	Significance[a]	ns	****	ns	*	ns	ns	****	****	****	**
	Interaction	ns	****	**	ns	ns	ns	****	****	****	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test. [b] concentrations in mg/L

Table 3.4.3 (Continuation)

Year	Factor	Isoamyl alcohol	Ethyl caproate	Ethyl phenyl acetate	Ethyl decanoate	2-Phenethyl acetate	Diethyl succinate	Isoamyl acetate	Ethyl acetate	Ethyl heptanoate	Benzal-dehyde	Damasce-none	Decanoic acid
2011	**Crop Level**												
	Full	1958.8	1.01	0	0.024	10.5	7.2	5	223.2	0.292	0.557	0.103	2.4
	Half	1896.5	0.96	0.164	0.023	10.5	6.6	5	219.7	0	0.579	0.146	2.8
	Significance[a]	ns	ns	****	ns	ns	ns	ns	ns	****	ns	ns	ns
	Hang time												
	T0	2056.1 a	0.92 ab	0.246 a	0.017 b	10.5	6.8 ab	5	172.3 c	0 b	0 b	0.15 a	3.5 a
	T1	2130.8 a	1.18 a	0 b	0.031 a	10.5	5.3 b	5	223 b	0 b	0 b	0.128 a	3.3 a
	T2	1596 b	0.85 b	0 b	0.022 ab	10.6	8.6 a	5	269.1 a	0.438 a	1.7 a	0.095 b	0.98 b
	Significance[a]	*	****	****	****	ns	*	ns	****	****	****	**	***
	Interaction	ns	ns	****	ns	ns	ns	ns	***	****	ns	*	ns
2012	**Crop Level**												
	Full	1210.7	0.42	0.123	0.014	10.5	7.8	4.9	122.1	0.015	0.004	0.156	2.7
	Half	1357.8	0.68	0.183	0.021	10.5	5.6	6.1	124.8	0	0.029	0.104	3.0
	Significance[a]	ns	***	****	ns	ns	*	*	ns	****	****	ns	ns
	Hang time												
	T0	1363.6	0.59	0.244 b	0.014 b	10.5	3.4 c	4.9 b	127.8	0 b	0 b	0.152 a	3.3 a
	T1	1370.3	0.51	0 c	0.016 b	10.5	9.7 a	5.0 b	123.9	0 b	0 b	0.109 a	2.7 b
	T2	1036.1	0.53	0.246 a	0.024 a	10.5	7.2 b	7.2 a	117.0	0.031 a	0.066 a	0 b	2.4 b
	Significance[a]	ns	ns	****	****	ns	***	***	ns	****	****	***	*
	Interaction	ns	*	****	ns	**	*	*	ns	****	****	ns	**

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test. [b] concentrations in mg/L

Table 3.4.4 Impact of hang time and crop level treatments on the wine volatile compounds [b] of Cabernet Sauvignon 2011-2012

Year	**Factor**	Ethyl butyrate	1-Hexanol	Isobutyl alcohol	Ethyl caprylate	Phenyl ethyl alcohol	Octanoic acid	Diethyl acetal	Citronellol	Isoamyl alcohol	Ethyl caproate
2011	**Crop Level**										
	Full	0.05	60.0	----	14.0	34.5	7.4	623.8	---	4339.0	3.0
	Half	0.05	58.6	----	6.8	29.7	6.5	317.7	---	3463.3	2.1
	Significance[a]	ns	**	----	ns	***	**	ns	---	*	*
	Hang time										
	T0	0 b	60.9 a	-	13.6	31.4 b	7.8 a	1412.1 a	---	3465.3	2.2
	T1	0 b	59.2 b	-	7.5	36.2 a	7 b	0 b	---	4452.9	2.9
	T2	0.15 a	57.8 c	-	10.1	28.7 c	5.9 c	0 b	---	3785.2	2.5
	Significance[a]	****	****	-	ns	***	***	****	---	ns	ns
	Interaction	ns	**	-	ns	*	*	ns	---	ns	ns
2012	**Crop Level**										
	Full	0.13	56.0	706.4	1.1	21.9	5.6	233.7	0.107	645.9	0.66
	Half	0.13	55.7	717.3	1.2	22.5	5.7	0	0.119	530.0	0.51
	Significance[a]	ns	*	ns	ns	ns	ns	****	ns	ns	ns
	Hang time										
	T0	0.133 a	56.3 a	747.4 a	1.10	24.4 a	5.8 a	0 b	0.182 a	323.4 b	0.75 a
	T1	0.132 b	55.9 a	692.8 b	0.85	21.8 b	5.8 a	0 b	0.119 b	816.5 a	0.65 a
	T2	0.131 b	55.3 b	687.0 b	1.60	19.4 c	5.0 b	467.3 a	0 c	641.9 ab	0.24 b
	Significance[a]	**	**	**	ns	***	**	****	****	*	**
	Interaction	**	**	***	ns	ns	ns	****	ns	*	ns

[a]. *, **, ***, ****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test. [b] concentrations in mg/L

Table 3.4.4 (Continuation)

Year	**Factor**	Hexanoic acid	Ethyl decanoate	2-Phenethyl acetate	Diethyl succinate	Isoamyl acetate	1-Nonanol	Ethyl acetate	Ethyl heptanoate	Benzal-dehyde	Damas-cenone	Decanoic acid
2011	**Crop Level**											
	Full	0	0.271	10.7	15.7	8.9	0.59	486.9	0	0	0.697	6.3
	Half	3.4	0.068	10.6	12.8	5.3	0.13	351.1	0	1.52	0.057	4.7
	Significance[a]	****	ns	**	ns	ns	****	ns	----	****	ns	**
	Hang time											
	T0	5.1 a	0.340	10.6 b	22.3 a	10.5	0.25 c	263.3 c	0	0 b	1.026	7.1 a
	T1	0 b	0.074	10.6 ab	7 b	5.2	0.31 b	407.9 b	0	0 b	0.056	6.6 a
	T2	0 b	0.094	10.7 a	13.6 ab	5.5	0.51 a	585.7 a	0	2.28 a	0.046	2.8 b
	Significance[a]	****	ns	ns	*	ns	****	ns	----	****	ns	****
	Interaction	****	ns	ns	ns	ns	****	ns	----	****	ns	ns
2012	**Crop Level**											
	Full	8.6	0.029	7.8	5.1	4.9	0	131.3	0.0013	0.247	0	3.3
	Half	5.8	0.024	7.8	11.5	5.0	0.045	130.8	0.0064	0.329	0	2.6
	Significance[a]	****	ns	ns	*	ns	****	ns	**	*		ns
	Hang time											
	T0	8.0 a	0.017 b	10.4 a	6.1 b	5.1 a	0 b	125.2 b	0 b	0 b	0	3.3 a
	T1	8.0 a	0.028 ab	10.4 a	3.4 b	4.9 ab	0 b	140.9 a	0.0038 b	0 b	0	4.5 a
	T2	4.7 b	0.039 a	0 b	19.0 a	4.8 b	0.09 a	125.1 b	0.0098 a	1.15 a	0	0 b
	Significance[a]	****	*	****	*	*	****	*	**	****	---	**
	Interaction	****	ns	ns	*	ns	****	***	ns	**	---	ns

[a]. *,**,***,****,ns: Significant at p≤ 0.05, 0.01, 0.001, 0.0001, or not significant respectively. Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test. [b] concentrations in mg/L

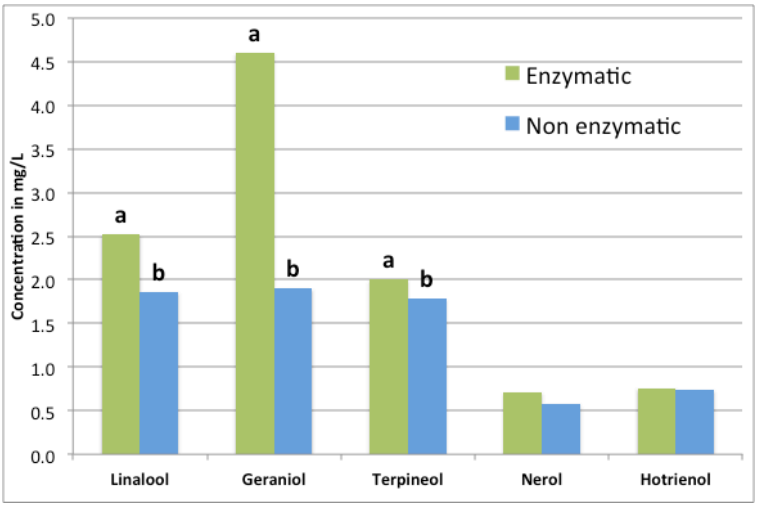

Figure 3.1 Effect of enzymatic treatment on the volatile compounds of Riesling must 2012*.

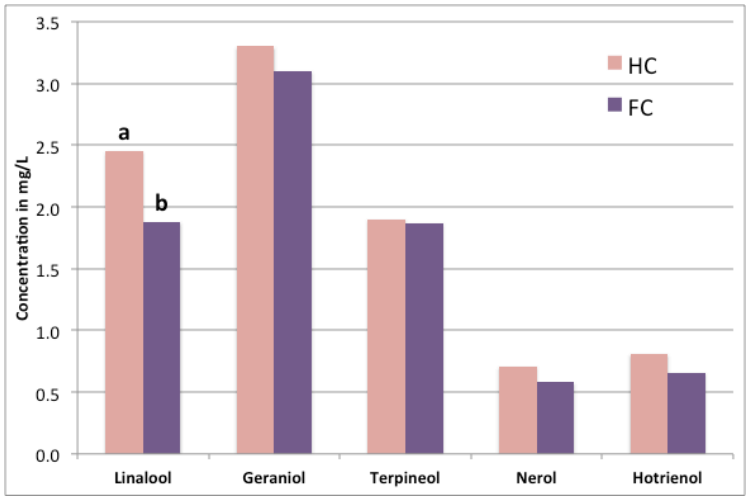

Figure 3.2 Effect of crop reduction on the volatile compounds of Riesling must 2012*.

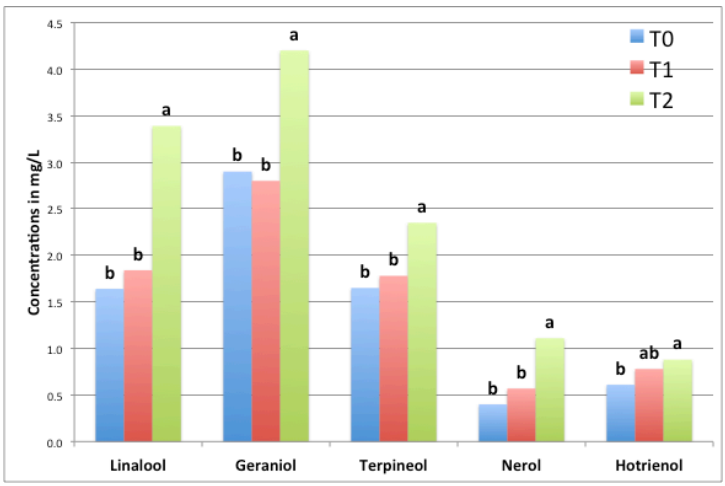

Figure 3.3 Harvest date effect on the volatile compounds of Riesling must 2012*.

*Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

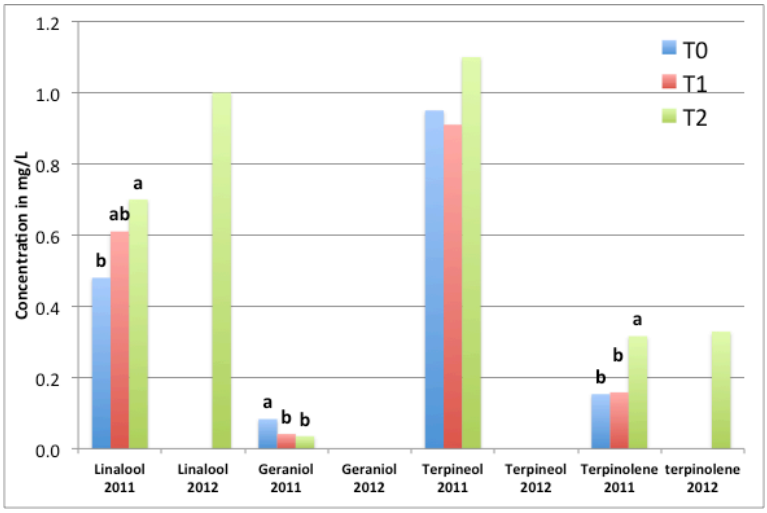

Figure 3.4 Harvest date effect on the volatile compounds of Riesling wine 1*.

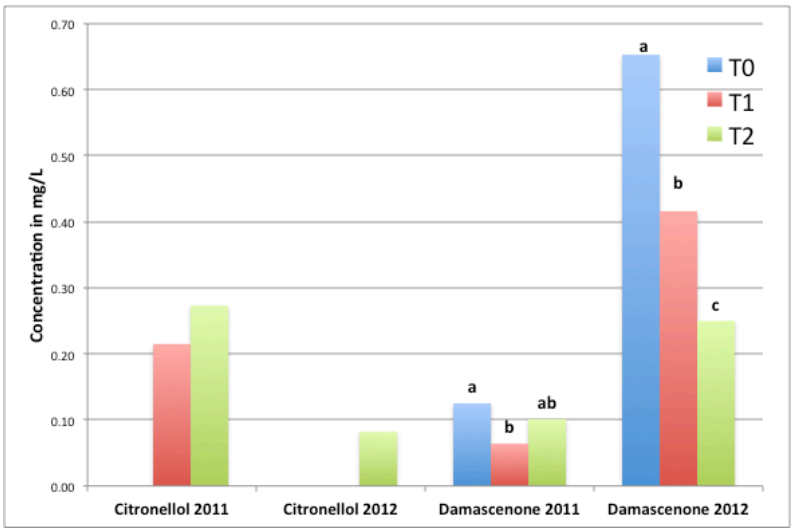

Figure 3.5 Harvest date effect on the volatile compounds of Riesling wine 2*.

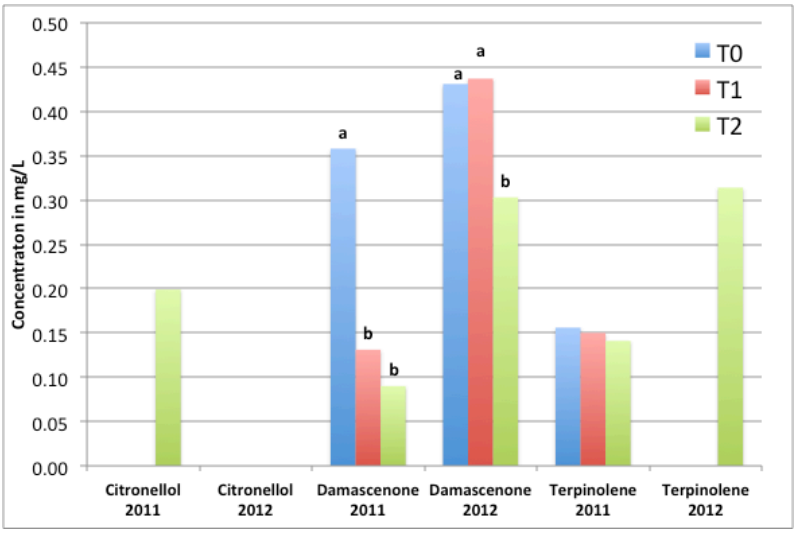

Figure 3.6 Harvest date effect on the volatile compounds of Pinot gris wine*.

*Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

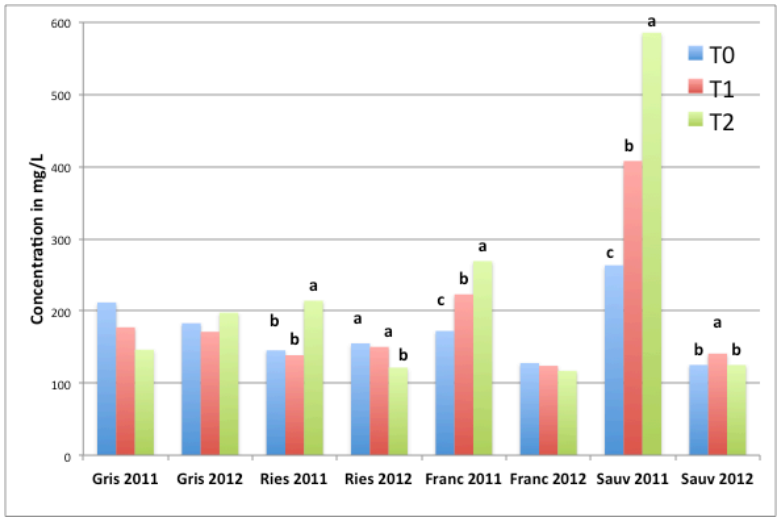

Figure 3.7 Harvest date effect on ethyl acetate*.

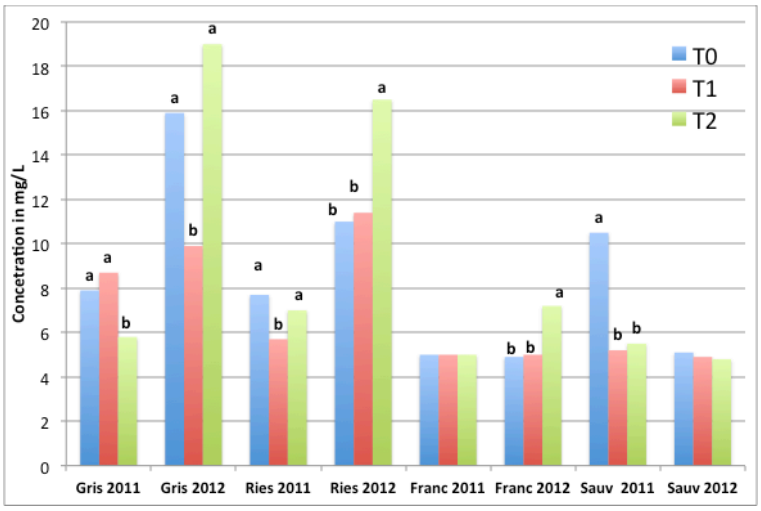

Figure 3.8 Harvest date effect on isoamyl acetate*.

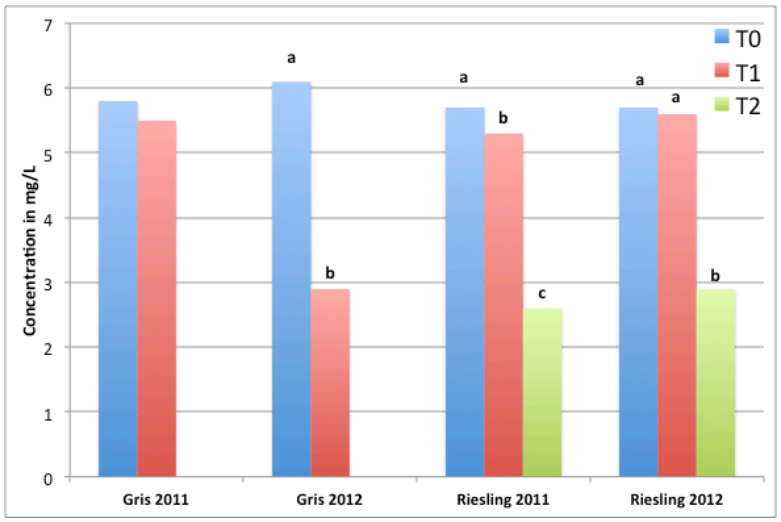

Figure 3.9 Harvest date effect on hexyl acetate*.

*Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test.

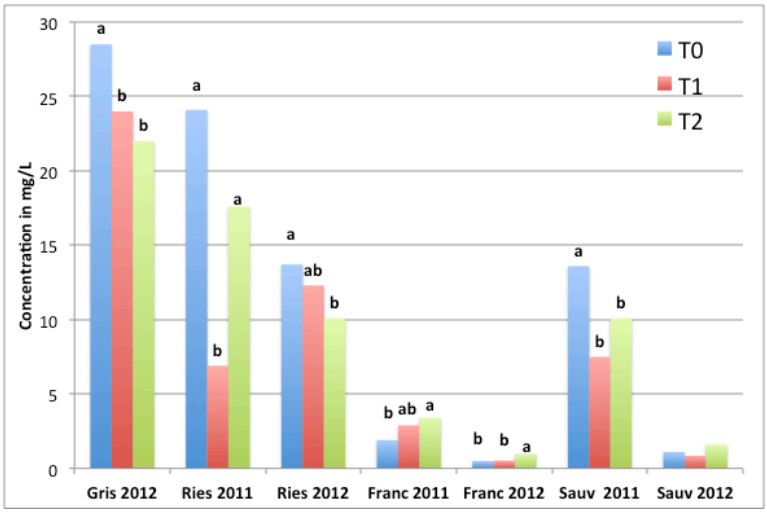

Figure 3.10 Harvest date effect on ethyl caprylate*.

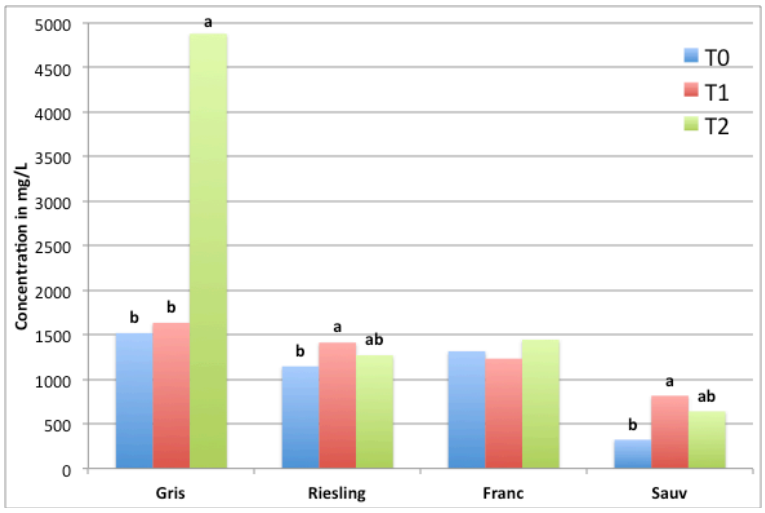

Figure 3.11 Harvest date effect on isoamyl alcohol 2012*.

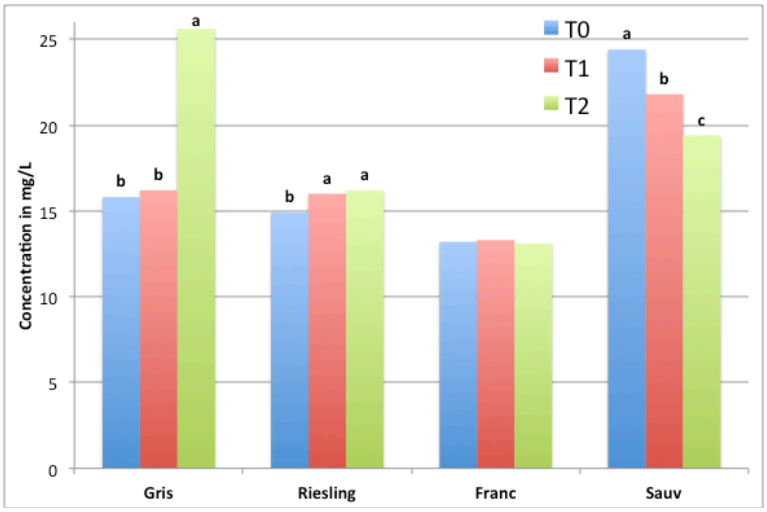

Figure 3.12 Harvest date effect on phenethyl alcohol 2012*.

*Mean values with same letters are not significantly different at p≤ 0.05 by Duncan´s multiple range test. [b] concentrations in mg/

Table 3.4.5 Odour activity values found in wine aromas, divided by crop level and hang time at each cultivar in 2011 and 2012.

Cultivar	YEAR	CROP LEVEL	HANG TIME	ETBU	1HEX	ISBU	HEXA	ECAR	PHEN	OCTA	DIAC	1HEP	LINA	CITR	GERA	ISOA	ECAP
Pinot Gris	2011	HC	T0	7.40	6.94		3.65		1.64	369						49.90	2046
		HC	T1	7.43	6.94		3.63		1.60	350						44.51	1924
		HC	T2	6.83	6.87	18.25			1.77	203	37286			1.97		46.70	686
		FC	T0	10.08	7.58	18.79	4.10		3.30	319						46.38	819
		FC	T1	7.60	6.91	17.34	3.66		1.69	407						50.81	2194
		FC	T2	7.14	6.88	18.86			1.78	226	31252			2.00		45.95	965
	2012	HC	T0	11.30			4.04	16891	1.58	745						53.14	2798
		HC	T1	8.44				8750	1.61	433						51.09	1568
		HC	T2	8.49				12121	1.59	367	188183					50.78	1382
		FC	T0	8.30	6.88		4.05	11621	1.57	752						48.22	2347
		FC	T1	9.08	6.89		3.81	14053	1.64	723						57.93	2736
		FC	T2	28.37	8.02			11922	3.51	3587	219					274.53	16571
Riesling	2011	HC	T0	7.48	6.98		3.88	14967	1.70	616			23.93		5.53	47.24	1533
		HC	T1	6.99	6.90		3.55	3796	1.68	228			28.10	2.44	2.73	43.06	399
		HC	T2	7.70		17.78	3.52	6007	1.71	330			21.51	2.40		44.15	858
		FC	T0	7.28	6.96		3.71	9151	1.64	424			14.01			40.53	1117
		FC	T1	6.91	6.90		3.52	3122	1.66	212			19.92	1.87		39.16	338
		FC	T2	7.66	6.82	20.10		11635	1.87	391	18891		33.77	3.05	2.30	52.30	1292
	2012	HC	T0				3.75	6623	1.47	612						38.82	1650
		HC	T1				3.79	6474	1.60	656						50.04	1930
		HC	T2	8.05	6.82			3860	1.57	373			35.87			37.95	945
		FC	T0	7.90	6.94		3.85	7121	1.51	778						37.64	1866
		FC	T1	8.80	6.90		3.73	5789	1.59	560						44.20	1325
		FC	T2	7.81	6.91	17.57	3.83	6214	1.67	419		0.0175	45.08	1.63		46.78	1683

Blank cells represent no aroma detected. Abbreviations for compounds: ETBU: Ethyl butyrate; 1HEX: 1-Hexanol; ISBU: Isobutyl alcohol; HEXA: Hexyl acetate; ECAR: Ethyl caprylate; PHEN: Phenyl ethyl alcohol; OCTA: Octanoic acid; DIAC: Diethyl acetal; 1HEP: 1-Heptanol; LINA: Linalool; CITR: Citronellol; GERA: Geraniol; ISOA: Isoamyl alcohol; ECAP: Ethyl caproate Decanal was undetected (data not shown).

Table 3.4.5 (Continuation)

Cultivar	YEAR	CROP LEVEL	HANG TIME	NONA	HEXA	EPHE	EDEC	TERP	1OCT	2PHE	DISU	IACT	ETAC	EHEP	BENZ	DAMA	DACA
Pinot Gris	2011	HC	T0		4.33		1.12			43.34	0.029	285	19			1716	2.08
		HC	T1		3.97	0.116	1.32			44.40	0.026	273	22.6			1493	2.07
		HC	T2		3.51	0.114	0.40			42.58		182	18			994	0.85
		FC	T0		16.22		4.87			48.18		741	37.5			12633	1.55
		FC	T1		4.46	0.116	1.38			44.82		306	24.7			3738	2.75
		FC	T2		3.40	0.116	0.70			42.90		203	21			2603	0.77
	2012	HC	T0		7.35		1.31		1.05	45.05		582	27.1			12313	4.36
		HC	T1		5.18		0.92		1.43	42.90		252	23.6			11783	2.93
		HC	T2		5.15		1.32		0.88	42.99	0.017	231	27.4		1.820	7924	1.33
		FC	T0		5.82		0.86		0.34	44.44		476	21.7			4928	4.29
		FC	T1		5.47		1.05		0.72	44.12		407	22			5715	4.31
		FC	T2		37.34	0.198	7.80		2.56	54.40	0.044	1036	86.8		10.727	4192	1.10
Riesling	2011	HC	T0		5.71	0.115	1.33	3.87		43.27		282	20.2			2560	2.66
		HC	T1		3.45	0.112	0.44	3.61		42.95		202	18.6			1087	0.85
		HC	T2		3.72	0.113	0.91	3.78		43.05		225	26.3			1864	2.15
		FC	T0		4.19	0.115	0.9	3.71		43.01		232	18.6			2422	1.85
		FC	T1	28.87	3.15	0.114	0.28	3.68		42.56		180	18.4			1493	0.86
		FC	T2		3.97	0.119	3.03	5.02		43.28		243	30.9			2194	3.14
	2012	HC	T0		6.26		0.43			43.07		377	21.4			12002	3.21
		HC	T1		4.93		0.38			44.36		416	19.4			3632	4.20
		HC	T2		3.53	0.111	0.26			44.26		356	17.3	0.116	0.274	4444	1.09
		FC	T0		6.36		0.51			43.33		357	20			14104	4.35
		FC	T1		5.29		0.47			44.09		342	20.6			13022	2.41
		FC	T2		4.27		0.4			44.88		742	15.1	0.133	0.881	5546	1.31

Blank cells represent no aroma detected. Abbreviations for compounds: NONA: Nonyl aldehyde; HEXA: Hexanoic acid; EPHA: Ethyl phenyl acetate; EDEC: Ethyl decanoate; TERP: Terpineol; 1OCT: 1-Octanol; 2PHE: 2-Phenethyl acetate; DISU: Diethyl succinate; IACT: Isoamyl acetate; ETAC: Ethyl acetate; EHEP: Ethyl heptanoate; BENZ: Benzaldehyde; DAMA: Damascenone; DECA: Decanoic acid.

Table 3.4.5 (Continuation)

Cultivar	YEAR	CROP LEVEL	HANG TIME	EBUT	1HEX	ISOB	ECAR	PHEN	OCTA	DIAC	1HEP	DECA	CITR	ISOA	ECAP
C. Franc	2011	HC	T0	6.70	7.05	18.34	1205	2.21	123				2.04	64.17	193
		HC	T1	6.85	6.99	21.36	1336	2.29	120				2.31	72.73	220
		HC	T2	6.90		15.82	1433	1.79	97	18869				52.75	162
		FC	T0		7.07	15.82	694	2.14	114					72.91	175
		FC	T1		6.96	15.82	1580	2.05	113					69.32	251
		FC	T2	6.90		15.82	1933	1.85	101	18845				53.65	177
	2012	HC	T0	6.60	6.95	18.38	257	1.32	118		0.0171		1.84	43.77	137
		HC	T1	6.65	6.90	16.97	283	1.35	127					45.58	120
		HC	T2	6.55		16.86	665	1.31	102	18664	0.0167			47.01	156
		FC	T0	6.60	6.97	17.26	238	1.31	116					47.14	100
		FC	T1	6.65	6.82	18.46	251	1.31	113			29.86	1.79	45.77	85
		FC	T2	6.55		17.73	312	1.31	105	18677	0.0166			22.06	56
C. Sauv-ignon	2011	HC	T0	6.50	7.41		1773	2.76	139	19060				101.47	280
		HC	T1	6.50	7.44		3952	3.60	139					139.07	578
		HC	T2	7.20	7.13		4428	2.56	111					105.78	403
		FC	T0	6.50	23.35		94484	3.52	1632	37426				260.02	1285
		FC	T1	6.50	7.35		3509	3.63	142					157.79	587
		FC	T2	7.30	7.32		5657	3.18	127					146.57	611
	2012	HC	T0	6.70	7.07	19.54	525	2.50	113			10.10	1.81		134
		HC	T1	6.55	6.97	17.73	410	2.18	124			9.90	1.37	32.87	134
		HC	T2	6.55	6.83	15.82	971	1.97	96					21.36	10
		FC	T0	6.60	7.01	17.83	574	2.38	120			10.00	1.83	21.56	165
		FC	T1	6.55	7.00	16.91	442	2.17	109				1.01	21.57	127
		FC	T2	6.60	7.01	18.53	634	1.92	103	18691				21.43	86

Blank cells represents not aroma detected. Abbreviations for compounds: EBUT: Ethyl butyrate 1HEX: 1-Hexanol; ISOB: Isobutyl alcohol; ECAR: Ethyl caprylate; PHEN: Phenyl ethyl alcohol; OCTA: Octanoic acid; DIAC: Diethyl acetal; 1HEP: 1-Heptanol; DECA: Decanal; CITR: Citronellol; ISOA: Isoamyl alcohol; ECAP: Ethyl caproate. Hexyl acetate, linalool, and geraniol were not detected (data not shown).

Table 3.4.5 (Continuation)

Cultivar	YEAR	CROP LEVEL	HANG TIME	HEXA	EPHE	ECAP	2PHE	DISU	IACT	NON	ETAC	EHEP	BENZ	DAMA	DECA
C. Franc	2011	HC	T0		0.113	0.115	42.14	0.029	171		17.8			3616	0.27
		HC	T1			0.160	42.16	0.027	167		29.1			3209	0.24
		HC	T2			0.070	42.16	0.043	167		40.9		1.135		0.05
		FC	T0			0.055	42.15	0.039	166		28.1			2389	0.20
		FC	T1			0.150	42.14	0.026	164		30.3			1926	0.19
		FC	T2			0.150	42.24	0.043	166		30.8	0.438	1.093	1891	0.08
	2012	HC	T0		0.112	0.090	42.12	0.017	166		17.3			3503	0.23
		HC	T1			0.100	42.09	0.035	167		15.9			2756	0.22
		HC	T2			0.125	42.13	0.033	319		17.3		0.075		0.12
		FC	T0			0.055	42.08	0.016	162		16.4			2558	0.21
		FC	T1			0.055	42.10	0.062	165		17.2			1609	0.14
		FC	T2		0.113	0.115	42.11	0.039	160		13.9	0.031	0.010		0.20
C. Sauvig-non	2011	HC	T0	3.42		0.180	42.33	0.112	167		30.5			1317	0.38
		HC	T1			0.395	42.55	0.012	171		47.1			1140	0.42
		HC	T2			0.440	42.48	0.069	187	77	62.9		2.975	944	0.14
		FC	T0			3.220	42.58	0.111	532	100	780			39769	11.30
		FC	T1			0.345	42.51	0.058	178	126	61.7			1121	0.46
		FC	T2			0.500	42.78	0.066	181	126	93.3			912	0.24
	2012	HC	T0	2.55		0.080	41.96	0.029	173		18				0.21
		HC	T1	2.58		0.135	41.97	0.018	164		19.4	0.004			0.25
		HC	T2			0.160		0.161	157	36	13.7	0.007	0.859		
		FC	T0	2.79		0.090	41.96	0.032	167		15.4				0.23
		FC	T1	2.76		0.150	41.96	0.016	162		18.1				0.35
		FC	T2	3.16		0.225		0.030	164		19.7	0.003	0.646		

Blank cells represent no aroma detected. Abbreviations for compounds: HEXA: Hexanoic acid; EPHE: Ethyl phenyl acetate; ECAP: Ethyl caproate; 2PHE: 2-Phenethyl acetate; DISU: Diethyl succinate; IACT: Isoamyl acetate: NON: 1-Nonanol; ETAC: Ethyl acetate; EHEP: Ethyl heptanoate; BENZ: Benzaldehyde; DAMA: Damascenone; DECA: Decanoic acid. Nonyl aldehyde, terpineol, 1-octanol were not detected

Table 3.5 Volatile standards for quantification

Compound	CAS #	RT [a]	m/z Ions	Calibration ranges mg/L	r^2	Odour quality [b]	Odour threshold mg/L [c]	Group [d]
Ethyl acetate	141-78-6	6.7	43, 44, 42	500, 250, 100	0.999	Pineapple	7.5	Esters
Isobutyl alcohol	78-83-1	9.1	43, 42, 41	5000, 2500, 1000	0.975	Wine, solvent, bitter	40	Alcohols
Ethyl butyrate	105-54-4	12.6	71, 73, 70	5, 1.5, 0	0.998	Apple	0.02	Esters
Isoamyl acetate	123-92-2	15.7	43, 42, 44	50, 10, 1	0.972	Banana	0.03	Esters
1-Hexanol	111-27-3	18.4	56, 55, 57	100,75,50	0.988	Resin, flower, green	8	Alcohols
Hexyl acetate	142-92-7	21	43, 42, 44	100,50,10	1	Fruit, herb	1.5 (1)	Esters
Benzaldehyde	100-52-7	23.1	106, 105, 107	10, 1, 0.1	0.998	Almond, burnt sugar, cherry, pistachio	1.53 (3)	Carbonyl aldehyde
Ethyl heptanoate	106-30-9	25.3	88, 97, 89	50, 10, 1	0.999	Fruit	2 (6)	Esters
Terpinolene	586-62-9	24.5	93, 136, 121	10, 1, 0.1	0.999	Pine, plastic	ND	Hydro-carbons
Ethyl caprylate	106-32-1	29.35	88, 101, 73	100, 50, 10	0.988	Fruit, fat	0.002	Esters
1-Nonanol	143-08-8	30.1	56, 55, 57	50, 10, 1	0.998	Fat, green	0.005 (2)	Alcohols
Phenyl ethyl alcohol	60-12-08	33	91, 92, 93	100, 50, 10	0.986	Honey, spice, rose, lilac	10	Alcohols
Octanoic acid	124-07-2	35	60, 61, 59	50, 10, 1	0.978	Sweat, cheese	0.05 (4)	Acids
Diethyl succinate	123-25-1	30.5	101, 129, 73	50, 10, 1	0.992	Wine, fruit	200 (1)	Esters
Diethyl acetal	105-57-7	9.5	45, 73, 103	5000, 2500, 1000	0.999	Fruit, cream	0.05	Acetates
1-Heptanol	111-70-6	22.3	70, 41, 56	50, 10, 1	0.999	Chemical, green	98.3 (2)	Alcohols
Linalool	78-70-6	26.9	71, 72, 70	50, 10, 1	0.971	Flower, lavender	0.0252 (4)	Alcohols
Decanal	112-31-2	30.05	57, 55, 56	50, 10, 1	0.999	Soap, orange peel, tallow	0.01 (5)	Carbonyl aldehyde
β-Citronellol	106-22-9	32.7	69, 68, 70	10, 1, 0.1	0.972	Rose	0.1	Alcohols
Geraniol	106-24-1	34	69, 68, 70	50, 10, 1	1	Rose, geranium	0.03	Alcohols
Damascenone	23726-93-4	36.2	69, 121, 190	0.5, 0.1. 0	0.991	Apple, rose, honey	0.00005	Noriso-prenoids
Isoamyl alcohol	123-51-3	12.8	41, 42, 43	5000, 2500, 1000	0.951	Whiskey, malt, burnt	30	Alcohols
Ethyl caproate	123-66-0	19.7	88, 99, 101	50, 10, 1	0.999	Apple peel, fruit	0.005	Esters
Nonyl aldehyde	124-19-6	25.9	57, 56, 55	50, 10, 1	0.999	Fat, citrus, green	0.015 (5)	Carbonyl aldehyde
Hexanoic acid	142-62-1	30.5	60, 61, 59	100, 50, 10	0.973	Sweat	3	Acids
Ethyl phenyl acetate	101-97-3	33.6	91, 92, 89	10, 1, 0.1	0.994	Fruit, sweet	4.36 (7)	Acetates
Ethyl decanoate	110-38-3	35.8	88, 89, 85	10, 1, 0.1	0.994	Grape	0.2 (4)	Esters
1-Octanol	111-87-5	26.2	56, 55, 57	50, 10, 1	0.991	Chemical, metal, burnt	0.19 (2)	Alcohols
α-Terpineol	98-55-5	31.4	59, 60, 61	50, 10, 1	0.996	Oil, anise, mint	0.25 (4)	Alcohols
2-Phenethyl acetate	103-45-7	33.9	104, 105, 103	100, 50, 10	0.966	Rose, honey, tobacco	0.25	Acetates
Decanoic Acid	334-48-5	36.7	73, 71, 74	100, 50, 10	0.974	Rancid, fat	15	Acids

[a]Retention time. [b] Odor perception from Flavornet database (flavornet.org). [c] Odor thresholds obtained from Guth (1997) determined in water/ethanol (90+10, w/w). Others from; (1) Etievant (1991) determined in 12% water/ethanol mix, (2) Ahumed (1978), (3) Keith and Powers (1968), (4) Ferreira et al (2000) determined in synthetic wine 11% v/v ethanol, 7 g/L glycerine, 5 g/L tartaric acid and pH adjusted to 3.4, (5) Culleré et al (2004) determined in 10% water/ethanol with 5 g/L tartaric acid at pH 3.2, (6) Burdock 2010. (7) Ruth (1986) [d] Base on classification of Rapp (1998). ND: data not available